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Environment Canada Environnement Canada

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Journal of the Fisheries Research Board of Canada

Growth and Cell-Size Distribution of Marine Planktonic Algae in Batch and Dialysis Cultures

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PRAKASH, A., L. SKOGLUND, B. RYSTAD, AND A. JENSEN. 1973. Growth and cell-size distribution of marine planktonic algae in batch and dialysis cultures. J. Fish. Res. Board Can. 30: 143–155.

An extended exponential growth phase and a higher maximum population characterized growth of planktonic algae in a dialysis system compared with that in a batch system. Algal cells grown in a dialysis culture had higher chlorophyll content and a larger average cell size than those grown in a batch culture. In both types of culture, changes in cell-size distribution were related to the phases of the growth cycle with maximum cell-size during the stationary phase. Various interactions of the component reactions of photosynthesis leading to changes in growth pattern and cell-size distribution are discussed.

PRAKASH, A., L. SKOGLUND, B. RYSTAD, AND A. JENSEN. 1973. Growth and cell-size distribution of marine planktonic algae in batch and dialysis cultures. J. Fish. Res. Board Can. 30: 143–155.

Une phase prolongée de croissance exponentielle et un maximum de population plus élevé caractérisent la croissance d'algues planctoniques dans un système de dialyse, comparativement à celle d'un système en série. Les cellules algales cultivées par dialyse ont un contenu en chlorophylle plus élevé et une taille plus grande que les cellules cultivées en série. Dans les deux types de cultures, les variations de la distribution de la taille des cellules sont liées aux phases du cycle de croissance, la grandeur maximale se rencontrant durant la phase stationnaire. Nous examinons diverses interactions des composantes de la photosynthèse aboutissant à des variations de croissance et de distribution de grosseur des cellules.

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MUCH of the information on growth kinetics of planktonic algae is based on observations carried out on *Chlorella* grown in the laboratory either in batch cultures (Myers 1953; Tamiya et al. 1953; Sorokin 1957; Williams 1971) or in continuous cultures (Phillips and Myers 1954; Sorokin and Krauss 1959; Tamiya 1964). However, reviews by Krauss (1958), Strickland (1960), Fogg (1965), and Eppley and Strickland (1968) have been particularly enlightening in their treatment of kinetics and physiology of growth processes in other freshwater and marine planktonic algae.

Growth of planktonic algae in cultures of unrenewed or limited volume generally follows the typical growth curve of microbial populations (Monod 1949) with its characteristic lag, exponential, declining or retardation, and stationary phases, each recognizable not only by changes in growth velocity, but also by alterations in cellular metabolism that accompany growth. Such a batch culture is typified by cellular heterogeneity in terms of physical, chemical, and metabolic attributes. The cessation of growth in a batch culture is explained largely on the basis of exhaustion of nutrients, inadequate illumination due to self-shading, and accumulation of growth inhibiting metabolic products. These limitations are to a considerable extent overcome in "continuous culture" where the algal cells can be maintained in the actively dividing state by regulation of the input of nutrients and output of growth products.

Several of the desirable features of a continuous culture are incorporated in a dialysis culture system that was originally designed to grow bacterial populations (Schultz and Gerhardt 1969) and was first successfully applied to growing a freshwater alga *Scenedesmus* by Trainor (1965). Recently dialysis culture techniques have been extended to growing a number of marine phytoplankton species (Jensen et al. 1972). The dialysis culture system employs exchange dialysis of nutrients and diffusible metabolites through a semipermeable membrane to obtain a high population of actively dividing cells. The production of such cells in a dialysis culture is

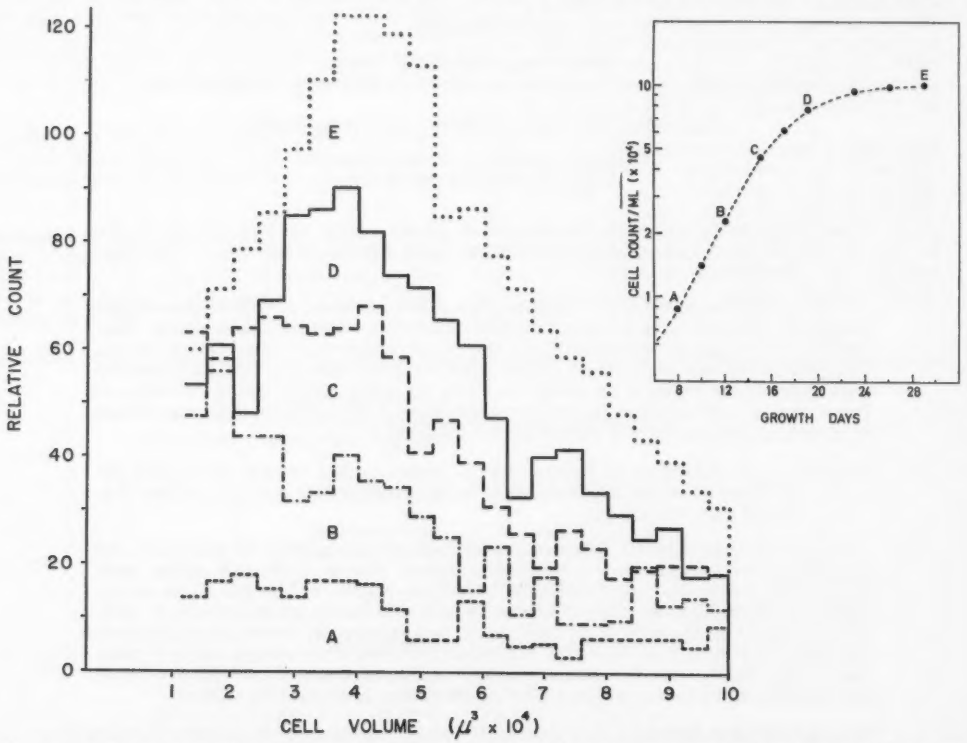

FIG. 1. Cell-size distribution of *Gonyaulax tamarensis* during the various stages of its 29 days growth in a batch culture recorded with an automatic particle size distribution analyzer.

maintained as long as the rate of exchange of chemicals across the membrane is constant, and the culture growth is not affected by density-dependent factors. If these conditions are not satisfied the growth pattern of the culture approaches that of a batch culture, differing from the latter only in an extended exponential and an elevated stationary phase.

During the various phases of the conventional growth cycle of an algal population the synthetic activity is channeled into different pathways. Thet production of new cells of rather uniform size and composition dominates the exponential growth phase implying that all the component reactions of the synthesis are running at the same rate when averaged over a full generation. During the declining and stationary phases, the rate of formation of new cells is slowed down considerably in relation to the rate of production of certain cellular components. If this situation persists for some time an increase in cell size and/or a release of storage products into the surrounding medium must take place. Since growth of algal populations may involve not only numerical increase of cells, but also variations in cell volume, changes in the cell-size distribution during the growth cycle become important in kinetic studies. With the introduction of electronic particle counting and sizing techniques (Maloney et al. 1962; Parsons 1965; Sheldon and Parsons 1967) it is now possible to follow growth and cell-size changes in an algal culture with considerable ease and precision. The present paper

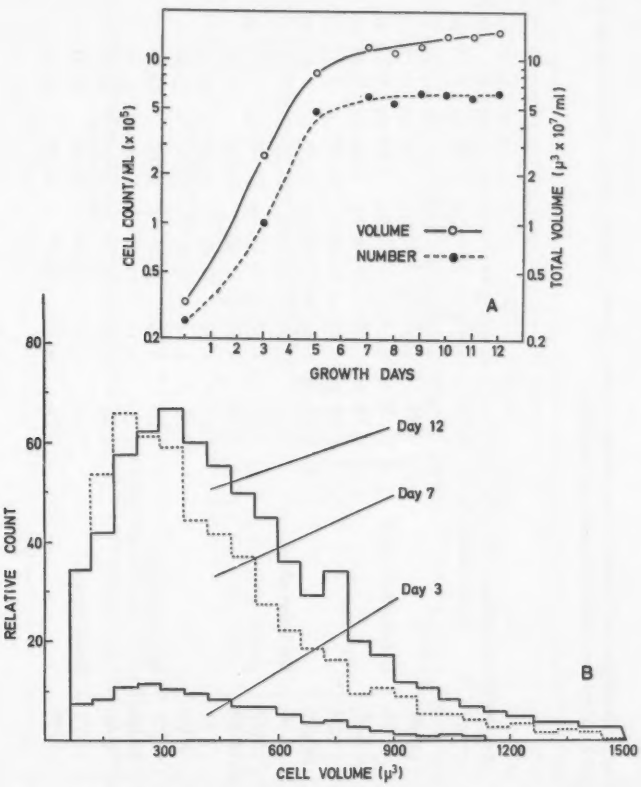

FIG. 2. Growth and cell-size distribution of *Skeletonema costatum*. A, Growth curves based on changes in cell numbers and cell volumes; B, Size distribution as a function of cell volume during exponential (day 3), declining (day 7) and stationary (day 12) growth phases recorded with an automatic particle size distribution analyzer.

TABLE 1. Cell-size distribution of *Cyclotella nana* during its entire growth in batch culture. Biomass in each cell-size group is given as volume ($\mu^3 \times 10^7$) per milliliter. The enclosed area represents the growing population.

Growth days	Counts/ml ($\times 10^6$)		Cell-size group								Total vol/ml ($\mu^3 \times 10^7$)
		Mean vol. (μ^3) Equiv. diam. (μ)	23.8 3.57	47.4 4.49	94.7 5.66	189 7.12	379 8.98	758 11.3	1520 14.3	3030 18.0	
2	0.69		0.49	1.07	3.55	1.04	0.19	0.16	0.07		6.57
3	2.16	Exponential phase	0.69	4.37	9.77	1.81	0.29	0.22	0.14	0.10	17.39
4	3.62		0.78	6.05	17.46	3.62	0.49	0.38	0.29	0.29	29.36
6	4.60		0.92	6.43	24.80	10.78	1.07	0.41	0.33	0.10	44.84
7	5.50		0.85	6.98	27.61	13.55	1.53	0.44	0.29	0.19	51.44
8	6.20	Declining phase	1.16	6.06	32.11	17.31	2.53	0.48	0.29	0.29	60.23
9	6.70		1.13	5.52	34.72	25.60	5.23	0.84	0.33	0.19	73.56
11	7.60		1.12	4.47	35.02	39.35	11.87	2.43	0.72	0.29	95.27
13	7.95		1.57	4.83	34.05	46.69	21.23	3.90	0.97	0.29	113.53
14	8.00		1.55	4.81	32.01	52.07	22.90	5.54	1.50	0.29	120.67
15	8.03	Stationary phase	1.74	5.10	31.37	52.88	25.31	5.69	1.64	0.19	123.92
16	8.03		1.70	5.11	31.07	52.95	25.95	6.24	1.70	0.38	125.10
17	7.90		1.94	5.91	31.77	54.75	24.83	3.88	0.72	0.29	124.09
18	7.93	Death phase	2.13	6.72	29.80	50.01	21.34	3.28	0.34	0.10	113.72
19	7.40		2.31	6.76	28.70	48.44	20.66	3.88	0.29	0.10	110.56

TABLE 2. Cell-size distribution of *Thalassiosira* sp. during its growth in a batch culture. Biomass in each cell-size group is given as volume ($\mu^3 \times 10^7$) per ml. Enclosed area represents the growing population.

Growth days	Counts/ml ($\times 10^5$)		Cell-size group									Total vol/ml ($\mu^3 \times 10^7$)
		Mean vol. (μ^3)	11.8	23.8	47.4	94.7	189	379	758	1520	3030	
		Equiv. diam. (μ)	2.82	3.57	4.49	5.66	7.12	8.98	11.3	14.3	18.0	
2	0.79		0.58	0.38	0.34	0.28	0.55	0.76	0.52	0.22	0.06	3.69
5	2.40	Exponential	0.28	0.40	0.36	0.49	2.79	2.10	0.43	0.19	0.13	7.17
6	4.84		0.38	0.48	0.41	0.60	5.02	4.00	0.51	0.21	0.10	11.17
8	7.06		0.38	0.61	0.42	0.97	8.80	4.41	0.36	0.24	0.19	16.38
10	8.63	Declining	0.47	0.62	0.39	1.40	12.04	5.78	0.48	0.19	0.13	21.50
12	12.16		0.62	0.40	0.37	2.32	15.32	5.35	0.60	0.21	0.13	25.32
14	13.08		0.26	0.31	0.35	2.16	15.35	6.36	0.79	0.37	0.26	26.21
16	13.49	Stationary	0.27	0.31	0.33	1.95	15.09	7.98	0.85	0.37	0.35	27.50
20	13.10		0.27	0.32	0.33	1.34	14.37	10.82	0.89	0.41	0.37	29.12

examines growth characteristics and cell-size distribution of a number of marine phytoplankton species grown in batch and dialysis cultures and discusses their physiological and metabolic implications.

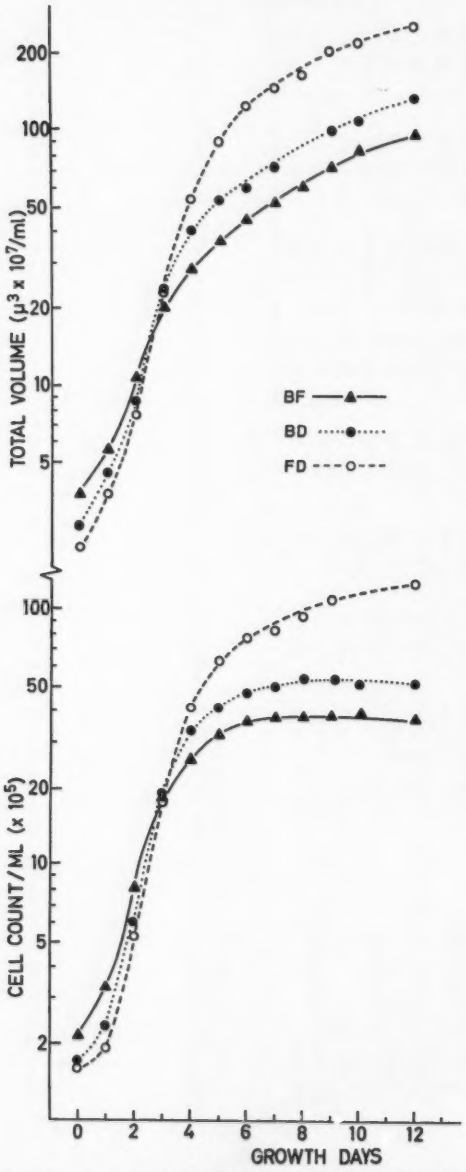

FIG. 3. Growth of *Cyclotella nana* in batch flask (BF), batch filter dialysis (BD), and continuous filter dialysis (FD) cultures.

Materials and Methods

ORGANISMS

The following organisms were used in the present study:

Skeletonema costatum (Grev.) Cleve. Clone Skel-5 kindly supplied by S. Myklestad, Norwegian Institute of Seaweed Research.

Cyclotella nana Hust. (=*Thalassiosira pseudonana* (Hust.) Hasle). Clone 3H kindly supplied by R.R.L. Guillard and J. H. Ryther, Woods Hole, Mass., U.S.A.

Thalassiosira sp. isolated from the Trondheimsfjord by S. Myklestad.

Phaeodactylym tricornutum Bohlin, also obtained from R.R.L. Guillard and J. H. Ryther.

Gonyaulax tamarensis Lebour, Portuguese strain, kindly supplied by L. Provasoli, Haskins Laboratories, New Haven, Conn., U.S.A.

CULTURE CONDITIONS

Batch flask cultures (BF) — Unialgal cultures of marine diatoms and dinoflagellates were grown axenically in 250-ml capacity Erlenmeyer flasks containing 50–100 ml medium. All cultures were incubated at 10 C under continuous illumination of about 4 klux provided by banks of cool-white fluorescent lamps. The inoculum was usually obtained from an exponentially growing parent culture unless otherwise specified. For diatoms half strength medium "*f*" of Guillard and Ryther (1962) and a completely synthetic medium ESWA (Prakash and Rashid 1968) containing 15 mg/liter sodium silicate was used. Dinoflagellates were grown in a modified marine synthetic medium ASP_7 (Prakash 1967).

Dialysis cultures — Since one of our objectives was to examine the growth of planktonic algae under different culture conditions, three variations of dialysis cultures were used.

(a) Continuous reservoir — Filter dialysis (FD). The culture flask assembly was similar to that described by Jensen et al. (1972). The upper growth chamber of the flask assembly contained approximately 150 ml medium and was separated from the lower chamber (350-ml capacity) by a Sartorius membrane-filter (pore size 1 μ). Cultures were grown in *f*/2 medium at 10–12 C under continuous illumination of 3.5–4 klux. Sterile medium was continuously pumped through the lower chamber at a rate of approximately 5 liters/day.

(b) Batch reservoir — Filter dialysis (BD). Culture conditions identical with FD but without continuous pumping of fresh medium in the lower chamber.

(c) Continuous reservoir — Bag dialysis. Cultures were grown in $\frac{3}{8}$-inch dialysis tubing tied at both ends to form bags that were suspended on a specially constructed rotor according to the arrangement outlined by Jensen et al. (1972). The rotor was

immersed in a flowing seawater tank through which water from 30 m depth was pumped at a rate of approximately 200 liters/day. The cultures were grown at 10 C under 3 klux light intensity with a light/dark cycle of 14/10 hr.

Determination of Growth and Cell-Size Distribution

Growth of algae was measured by regularly monitoring changes in cell numbers and cell volumes using a Coulter

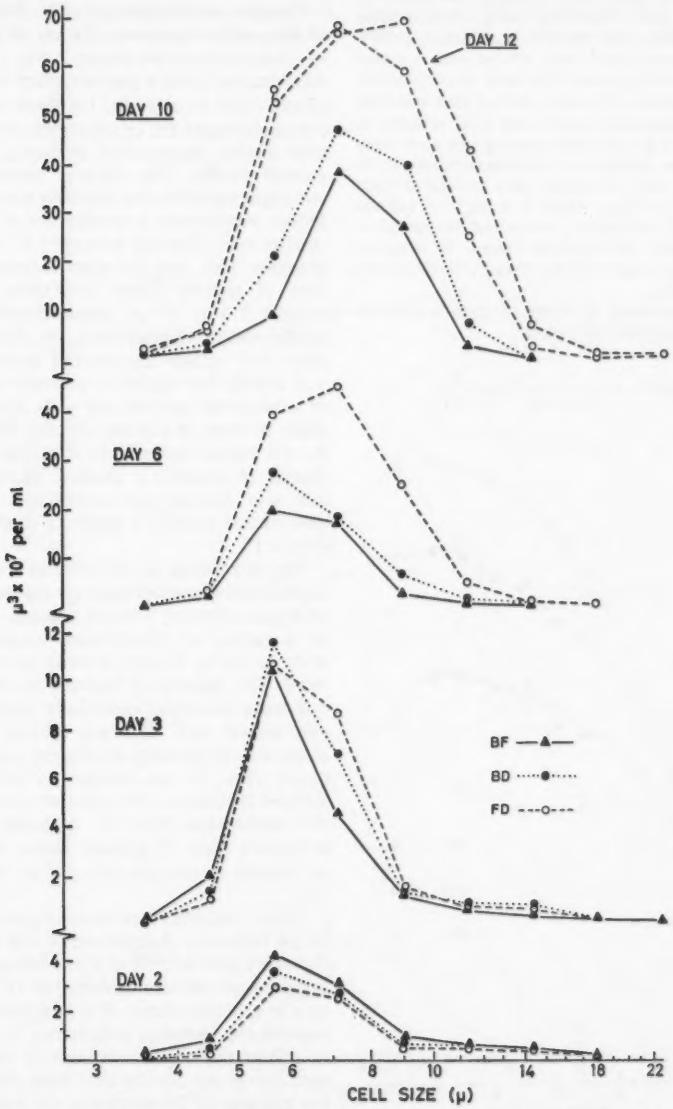

FIG. 4. Cell-size distribution of *Cyclotella nana* during various phases of its growth in BF, BD, and FD cultures. Cell size refers to equivalent spherical diameter.

counter (model B) fitted with a 100μ aperture tube and a model M volume converter. No coincidence corrections were made in cell counts since errors due to coincident passage of cells through the electrical field were always below 10% (see Sheldon and Parsons 1967).

Algal population was counted and scanned at 24 hr intervals for cell-size spectrum by changing the sensitivity factor by two for each succeeding size-group according to the method outlined by Sheldon and Parsons (1967). Size-frequency distributions were plotted with a model J particle size distribution analyzer used in conjunction with the Coulter counter. However, when greater accuracy and details were required, counts and total volumes in each of the 12 size groups (representing cell sizes from 2.52–40.3 μ) were plotted on a semilogarithmic scale. Total volume for each size-group was calculated from the expression, $V = NC_f v$, where V is the total volume (μ^3/ml culture), C_f represents a correction factor incorporating calibration and dilution values, N refers to number of cells/ml, and v is the mean cell volume in cubic microns (μ^3).

Growth was expressed in terms of growth constant (K) calculated using the expression

$$K_{10}\ (days)^{-1} = \frac{1}{(t_2 - t_1)} \log_{10} \frac{V_2}{V_1}$$

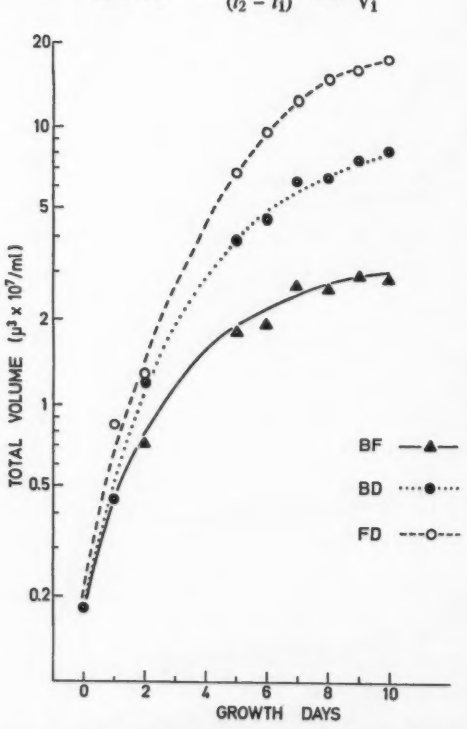

FIG. 5. Growth of *Skeletonema costatum* in BF, BD, a nd FD cultures.

where V_1 and V_2 represent cell volumes at times t_1 and t_2, respectively.

Results

BATCH CULTURES

Changes in cell numbers and cell-size distribution of *Gonyaulax tamarensis* during its 29 days growth in a batch culture are shown in Fig. 1. The inoculum was obtained from a parent culture in the stationary phase. After an extended lag phase of 4–6 days, the culture followed the characteristic pattern of growth with distinct exponential, declining, and stationary growth phases. The cell-size distribution showed two main modes during the early stages. One of these modes represented a population of newly formed smaller cells (average volume 1.8×10^4 μ^3, mean diameter 33μ), and the other represented a population of slightly larger and older cells (average volume 3.7×10^4 μ^3, mean diameter 41μ). These modes remained prominent and distinct during the early and middle exponential growth phase (Fig. 1,A and B) but tended to coalesce towards the end of exponential growth and were accompanied by a slight increase in average cell size (Fig. 1,C and D). As the culture moved into declining and stationary phases of growth, a marked increase in average cell size became noticeable and the frequency distribution showed a tendency to shift to the right (Fig. 1,E).

The increments in cell volume towards the post-exponential growth phase were also seen in a number of diatom cultures. Growth and cell-size progression in a culture of *Skeletonema costatum* monitored daily during its 12 days growth are shown in Fig. 2. While the pattern of increase in cell number and cell mass remained essentially similar during the exponential and declining phases, cell volumes increased significantly during the stationary growth phase when no net increase in cell numbers was noticed (Fig. 2A). The cell-size distribution during the exponential (day 3), declining (day 7), and stationary (day 12) growth phases showed distinct increments in cell size with age of the culture (Fig. 2B).

Table 1 incorporates data on growth and changes in the frequency distribution of cell volumes during the entire growth cycle of *Cyclotella nana*. By arranging data on cell-size distribution of a growing culture in a tabular form, it is possible not only to recognize the growing population of cells, but also to follow day to day changes in cell volumes for each size-group. Similar data have also been collected for cultures of *Thalassiosira* sp. isolated from the Trondheimsfjord (Table 2). In both species, during the exponential growth phase the populations were dominated by actively dividing smaller cells, but

with the onset of the declining phase of growth, a definite shift towards larger cell dimensions was initiated which reached its peak expression during the stationary growth phase. As the cultures approached the death phase, a gradual but significant decline in volume increments in large-sized cells became apparent and was often accompanied by modest increases in cell volumes in the smaller-sized cells. Table 1 shows such a situation. Here the increases in the two smallest cell-size groups (mean diameter 3.57 and 4.49 μ, respectively) during the death phase were more apparent than real because lysing and fragmentation of larger cells at this stage becomes fairly common and the Coulter Counter is unable to discriminate between small intact cells and fragments of other larger cells.

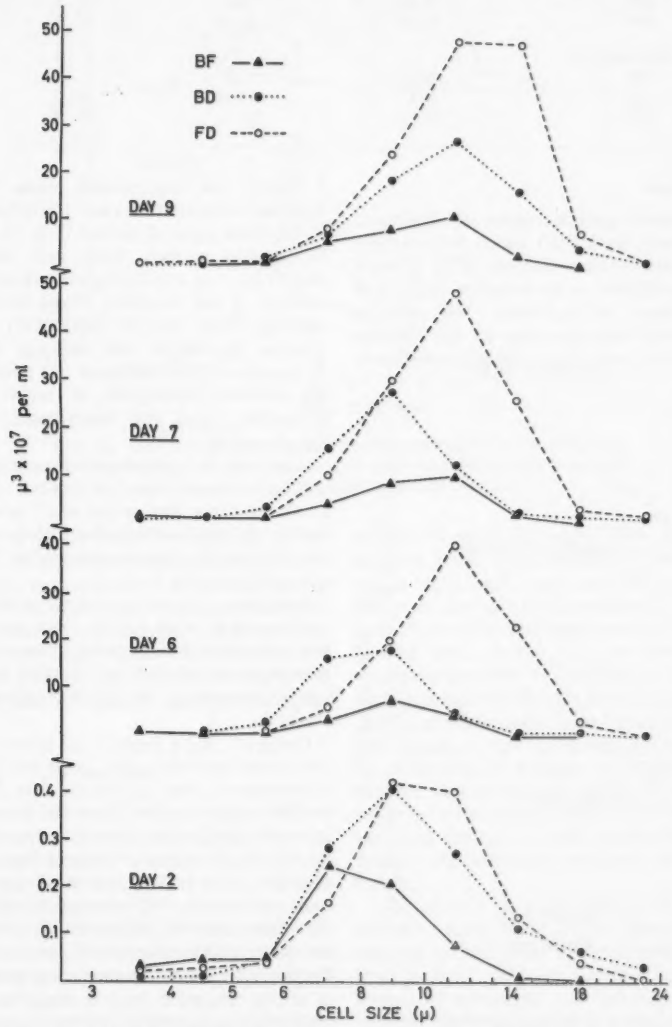

FIG. 6. Cell-size distribution of *Skeletonema costatum* during various phases of its growth in BF, BD, and FD cultures. Cell size refers to equivalent spherical diameter of a chain.

TABLE 3. Growth constants and chlorophyll concentrations during exponential growth of *Cyclotella nana* and *Skeletonema costatum* in BF, BD, and FD cultures.

	Growth constant K_{10} $(days)^{-1}$	Chlorophyll/cell ($\mu g \times 10^{-8}$)	Chlorophyll/μ^3 ($\mu g \times 10^{-10}$)
Cyclotella			
BF	0.28	3.5	3.2
BD	0.43	8.7	7.3
FD	0.48	21.3	16.3
Skeletonema			
BF	0.14	–	–
BD	0.17	41.6	15.9
FD	0.27	103.3	23.7

DIALYSIS CULTURES

Figure 3 presents growth curves of *Cyclotella nana* grown in batch flask (BF), batch filter dialysis (BD), and continuous filter dialysis (FD) cultures under similar conditions of temperature, light, and initial concentration of nutrients. The cell-size distribution curves corresponding to the various stages of growth in the three cultures are shown in Fig. 4.

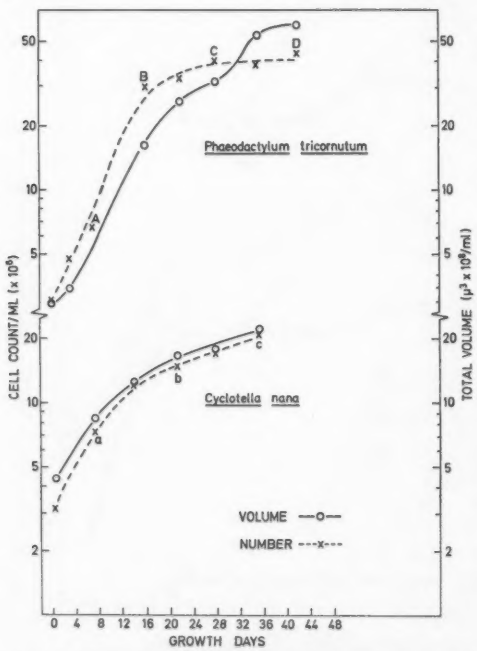
Phaeodactylum tricornutum
Cyclotella nana
VOLUME —o—
NUMBER ---x---
CELL COUNT/ML (x 10⁴)
TOTAL VOLUME (μ³ x 10⁹/ml)
GROWTH DAYS
0 4 8 12 16 20 24 28 32 36 40 44 48

FIG. 7. Growth of *Phaeodactylum tricornutum* and *Cyclotella nana* in bag dialysis cultures.

During the exponential phase the pattern of increase in cell numbers and cell volumes was similar in the three types of culture (Fig. 3), while the post-exponential growth phase was characterized by significant and continuing increases in cell volume relative to cell numbers. These features were also reflected in the cell-size distribution curves (Fig. 4). Volume increments and cell-size distribution of *S. costatum* in BF, BD, and FD cultures grown under identical conditions of initial nutrient concentration, light, and temperautre are shown in Fig. 5 and 6.

Although the overall growth patterns of *S. costatum* in the three types of cultures were essentially similar to those seen earlier in *C. nana*, each culture during the exponential growth showed considerable variation in its growth rate and in its chlorophyll content (Table 3).

While the cell-size increments in BF, BD, and FD cultures of *C. nana* and *S. costatum* become more pronounced with age, a trend towards an increase in average cell-size during the early stages of growth was characteristic of dialysis cultures only (Fig. 4 and 6).

Figures 7 and 8 present the growth and cell-size distribution patterns of *C. nana* and *Phaeodactylum tricornutum* in bag dialysis cultures. So long as the dialysis bags remained intact and the rate of dialysis was not significantly altered, a very dense population of algal cells was obtained. Such a population showed a reasonably constant cell number–cell mass relationship with age and its cell-size distribution also remained fairly uniform. These conditions are seen in the culture of *C. nana* (Fig. 7 and 8). On the other hand, the atypical growth pattern seen in the culture of *P. tricornutum* (Fig. 7 and 8) and reminiscent of growth pattern in a batch culture was largely due to changes in dialysis characteristics of the bag. The abrupt increase in cell volumes after 28 days of growth reflects sudden changes in

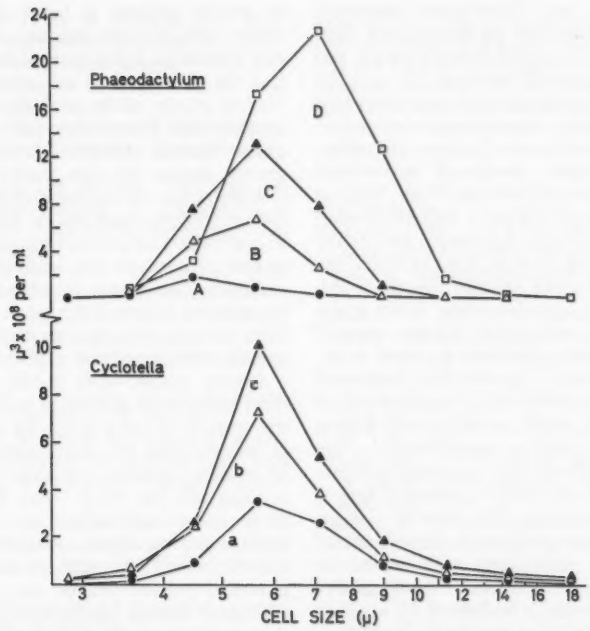

FIG. 8. Cell-size distribution of *Phaedactylum* and *Cyclotella* during the various stages of their growth in bag dialysis cultures as indicated in Fig. 7. Cell size refers to equivalent spherical diameter.

dialysis kinetics resulting from deterioration of the membrane and its subsequent replacement. As a rule the cellulose bags remained intact and transparent for about 3 weeks in the sea water. However, in more extended experiments the cultures were transferred to new bags approximately every 14 days to prevent loss of the culture and to avoid reduction in diffusion properties and light transparency.

Discussion

A batch culture is essentially a closed system where conditions are constantly changing and which result in metabolic heterogeneity of cells. Its functions are strictly time-dependent. A dialysis culture, on the other hand, represents an open system that favors preponderance of rapidly dividing cells and although such a culture develops in time, its functions may not be necessarily time-dependent. In several characteristics a dialysis culture is comparable to a continuous culture, but offers more possibilities for manipulation. For example, low exchange rate in the growth chamber and rapid replenishment of dilute nutrient solution in the reservoir make it possible to maintain a dense algal population at low nutrient level. In addition, diffusible metabolites which may inhibit cell growth are eliminated by diffusion through the membrane. Kinetics of algal growth in dialysis cultures have not been adequately studied but it seems that for the most part they approximate those of continuous cultures. Schultz and Gerhardt (1969) who have dealt with theoretical and quantitative aspects of bacterial growth in dialysis cultures, have proposed mathematical models to relate dialysis kinetics to growth kinetics. Such models may be equally applicable to algal growth provided relationship between rate of nutrient utilization and the rate of cell production can be determined with sufficient precision for different algal species.

The pattern of algal growth observed in batch cultures on one hand and in three types of dialysis cultures on the other in the present study, suggests that the final yield is a function of how open each system is. Although our BD and FD cultures represent progressively more open systems than an ordinary batch culture, they are far from a completely open system comparable to a fully continuous culture system. They are more like 'batch

reservoir–fermentor' and 'continuous reservoir–fermentor' cultures described by Schultz and Gerhardt (1969). Since the growth responses are allowed to accumulate in the culture chamber, BD and FD cultures represent a non-steady batch situation which differs from an ordinary non-dialysis batch culture in a prolonged exponential growth phase and consequently a much higher maximum population. While the growth patterns of batch and dialysis cultures remained generally similar, the latter tended to give steeper slopes of the exponential part of the growth curves than the former (Fig. 3). Since the main feature of the dialysis cultures compared with the batch culture was a prolongation of the exponential growth phase, we expected the two types of cultures to show differences only in final cell densities and not in the rate of growth. The differences seen in growth rates and cell-size distributions of exponentially growing algae in batch and dialysis cultures suggest that cells are metabolically more active in a dialysis system. The increased contents of chlorophyll in cells of dialysis cultures (Table 3) strengthens this impression. The growth pattern observed in bag dialysis cultures is reminiscent of continuous cultures, particularly with regard to cell number–cell mass relationship. In such cultures the growth does not seem to be limited by nutrient diffusion because of the large membrane area provided by dialysis bags. The cells continue to divide at first exponentially and later in a linear fashion till the growth becomes limited by reduced illumination due to selfshading and/or by deterioration of the dialysis membrane.

In connection with the dialysis cultures it should also be mentioned that stirring or agitation of cultures could be responsible for reducing the diffusion layer around each algal cell, thus enhancing the transport of nutrients to and removal of metabolic products from the cells on a microscopic level. Macroscopically it is possible that some inhibitory effect of cell metabolites (autoinhibition) may have been partly responsible for the differences in growth patterns between batch and dialysis cultures observed during exponential growth. Since the dialysis system is relatively more open than the batch system, the elimination of diffusible fractions of metabolites could have reduced the inhibitory effect in the former. Autoinhibition by metabolic products of algal cells accumulating in the medium has been noticed by several workers and dialysis techniques appear to be ideal for a critical study of this phenomenon in algal cultures.

In the causal sense cell-size changes in a growing culture of strictly autotrophic alga cannot be regarded as a direct function of age, although the results obtained in the present study show cell-size variations to be time-dependent. A comparison of growth patterns in batch and dialysis cultures clearly demonstrates that the enlargement of cells was associated with suppression of cell division and that the differences in cell size were related to the various phases of the growth cycle. In general the smaller cells dominated the exponential growth phase, whereas during the declining and stationary growth phases the cells tended to become larger. It appears that during the exponential growth phase the component reactions of biomass synthesis are in synchrony and are geared to production of daughter cells of more or less uniform size and chemical make-up. The change in cell size during the post-exponential period indicates not only a departure from this synchronous state but also reflects a fundamental shift in cellular metabolism.

Several studies have shown that the transition from exponential growth to linear or declining relative growth brought about by some limiting factor is accompanied by major alterations in synthesis of cellular material and other metabolic activities of algal cells (see Fogg 1965). Although the nature of synthetates may depend on conditions prevailing during photosynthesis, proteins are usually the major product of photosynthesis during the exponential growth, wheres, the synthesis of storage products, mainly carbohydrates, may dominate the post-exponential growth. Since cell-size increments become more pronounced during the declining and stationary phases of growth, the accumulation of storage products in some way appears to be related to the enlargement of algal cells. It has also been shown that extracellular release of storage products, particularly of carbohydrates is increased considerably during the terminal stages of growth of algal cultures (Guillard and Wangersky 1958; Curl and McLeod 1961; Marker 1965). This extracellular release of photosynthetic products may provide an alternative to otherwise enormous cells in an old culture.

Acknowledgments

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CURL, H., JR., AND G. C. MCLEOD. 1961. The physiological ecology of a marine diatom *Skeletonema costatum* (Grev) Cleve. J. Mar. Res. 19: 70–88.

EPPLEY, R. W., AND J. D. H. STRICKLAND. 1968. Kinetics of marine phytoplankton growth, p. 23–62. *In* M. R. Droop and E. J. Ferguson Wood [ed.] Advances in microbiology of the sea. Vol. 1. Academic Press, Inc., New York, N.Y.

FOGG, G. E. 1965. Algal cultures and phytoplankton ecology. The Univ. of Wisconsin Press, Madison, Wis. 126 p.

GUILLARD, R. R. L., AND J. H. RYTHER. 1962. Studies on marine planktonic diatoms. I. *Cyclotella nana* Hustedt and *Detonula confervacea* (cleve) Gran. Can. J. Microbiol. 8: 229–239.

GUILLARD, R. R. L., AND P. J. WANGERSKY. 1958. The production of extracellular carbohydrates by some marine flagellates. Limnol. Oceanogr. 3: 449–454.

JENSEN, A., B. RYSTAD, AND L. SKOGLUND. 1972. The use of dialysis culture in phytoplankton studies. J. Exp. Mar. Biol. Ecol. 8: 241–248.

KRAUSS, R. W. 1958. Physiology of the freshwater algae. Ann. Rev. Plant Physiol. 9: 207–244.

MALONEY, T. E., E. J. DONOVAN, JR., AND E. L. ROBINSON. 1962. Determination of numbers and sizes of algal cells with an electronic particle counter. Phycologia 2: 1–8.

MARKER, A. F. H. 1965. Extracellular carbohydrate liberation in the flagellates, *Isochrysis galbana* and *Prymnesium parvum*. J. Mar. Biol. Ass. U.K. 45: 755–772.

MONOD, J. 1949. The growth of bacterical cultures. Ann. Rev. Microbiol. 3: 371–394.

MYERS, J. 1953. Growth characteristics of algae in relation to the problems of mass culture, p. 37–54. *In* J. S. Burlew [ed.] Algal culture: from laboratory to pilot plant. Carnegie Inst. Wash. Publ. No. 600.

PARSONS, T. R. 1965. An automated technique for determining the growth rate of chain-forming phytoplankton. Limnol. Oceanogr. 10: 598–602.

PHILLIPS, J. N., JR., AND J. MYERS. 1954. Measurement of algal growth under controlled steady-state conditions. Plant Physiol. 29: 148–152.

PRAKASH, A. 1967. Growth and toxicity of a marine dinoflagellate, *Gonyaulax tamarensis*. J. Fish. Res. Board Can. 24: 1589–1606.

PRAKASH, A., AND M. A. RASHID. 1968. Influence of humic substances on the growth of marine phytoplankton: dinoflagellates. Limnol. Oceanogr. 13: 598–606.

SCHULTZ, J. S., AND P. GERHARDT. 1969. Dialysis culture of microorganisms: design, theory and results. Bacteriol. Rev. 33: 1–47.

SHELDON, R. W., AND T. R. PARSONS. 1967. A practical manual on the use of the Coulter counter in marine science. Coulter Electronics Sales Co., Canada. 66 p.

SOROKIN, C. 1957. Changes in photosynthetic activity in the course of cell development in *Chlorella*. Physiol. Plant. 10: 659–666.

SOROKIN, C., AND R. W. KRAUSS. 1959. Maximum growth rates of *Chlorella* in steady-state and in synchronous cultures. Proc. Nat. Acad. Sci. USA 45: 1740–1744.

STRICKLAND, J. D. H. 1960. Measuring the production of marine phytoplankton. Fish. Res. Board Can. Bull. 122: 172 p.

TAMIYA, H. 1964. Growth and cell division in *Chlorella*, p. 247–305. *In* E. Zeuther [ed.] Synchrony in cell division and growth. Interscience Publishers, Inc., New York, N.Y.

TAMIYA, H., E. HASE, K. SHIBATA, A. MITUYA, T. IWAMURA, T. NIHEI, AND T. SASA. 1953. Kinetics of the growth of *Chlorella*, p. 204–232. *Im* J. S. Burlew [ed.] Algal culture: from laboratory to pilot plant. Carnegie Inst. Wash. Publ. No. 600.

TRAINOR, F. R. 1965. A study of unialgal cultures of *Scenedesmus* incubated in nature and in the laboratory. Can. J. Bot. 43: 701–706.

WILLIAMS, F. M. 1971. Dynamics of microbial populations, p. 197–267. *In* B. C. Patten [ed.] Systems analysis and simulation in ecology. Vol. 1. Academic Press, Inc., New York, N.Y.

Effect of Season on Salt-Extractable Protein in Muscle from Trawler-Caught Cod and on its Stability During Frozen Storage

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CASTELL, C. H., AND D. M. BISHOP. 1973. Effect of season on salt-extractable protein in muscle from trawler-caught cod and on its stability during frozen storage. J. Fish. Res. Board Can. 30: 157–160.

Periodical tests made on 272 fillets from fresh, postrigor, trawler-caught cod have shown that there is a slight decrease in salt-extractable proteins during the spring spawning period (March, April, and May). This was accompanied by a similar slight decrease in the lipid content of the muscle.

When other fillets from these same lots of cod were frozen and stored at –3.3 C and –12 C, those from the fish caught during the spring spawning period tended to deteriorate faster, as indicated by the rate at which the EPN values decreased during storage. When the fish were stored at –26 C for up to 2 years, the EPN changes during storage were so retarded that seasonal differences in storage quality could not be observed.

CASTELL, C. H., AND D. M. BISHOP. 1973. Effect of season on salt-extractable protein in muscle from trawler-caught cod and on its stability during frozen storage. J. Fish. Res. Board Can. 30: 157–160.

Des essais effectués périodiquement sur 272 filets prélevés après rigor sur des morues fraîches, capturées au chalut, révèlent qu'il y a légère diminution des protéines extractibles au sel durant la fraie de printemps (mars, avril, et mai). Il y a également faible diminution de la teneur en lipides du muscle.

Lorsque d'autres filets provenant des mêmes lots de morues sont congelés et entreposés à –3.3 C et –12 C, les filets prélevés sur les morues capturées durant la fraie de printemps ont tendance à se détériorer plus rapidement, comme l'indique le taux auquel les valeurs d'EPN diminuent au cours de l'entreposage. Après un entreposage d'une durée allant jusqu'à 2 ans à –26 C, il y a un tel décalage dans les variations d'EPN que les différences saisonnières de qualité d'entreposage ne peuvent être décelées.

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CONSIDERABLE significance has been attached to the relationship between salt-extractable proteins and changes that occur in the quality and texture of stored frozen fish (Dyer 1951, 1967; Dyer et al. 1957; Love 1962; Ironside and Love 1958; Heen 1954; Luijpen 1957; Connell 1962, 1969). As the salt-solubility of the actomyosin decreases during storage the muscle has a tendency to become tough and fibrous; it loses its characteristic fresh-fish flavor and there is an increase in the amount of drip when it is defrosted.

At the time of death the amount of salt-extractable protein in cod muscle is usually 2.25–2.30%, expressed as percent extractable protein nitrogen (EPN) in the fillet. During the development of rigor mortis these values may decrease considerably. If the muscle has sufficient ATP, and if it is allowed to contract freely, during the development of rigor the EPN values may decrease as much as 50%, and then rise again to their initial values as the rigor is resolved. These immediate postrigor values remain at a fairly constant level for 6–8 days in well-iced fish (Dyer 1967).

In addition to these fluctuations that accompany the normal, post mortem changes in the fish muscle, there are also variations in EPN values that are the result of the physiological condition of the fish. Deliberate starvation depletes many of the components of the muscle, including both total and salt-extractable proteins (Love and Robertson 1967; Robertson et al. 1967). A similar but less drastic depletion occurs during prolonged periods of slack winter feeding; this is intensified if the slack feeding occurs when the fish are in the process of converting some of their body reserves into milt and roe (Ironside and Love 1958; Dambergs 1964). Ironside and Love (1958) and Bykov (1971) found that for codfish the initial (or postrigor) EPN values

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were lower in the late winter and early spring than during the remainder of the year.

In studies carried out in this laboratory we had occasion to measure the immediate postrigor EPN values of 272 cod that were caught at various times throughout the year. Similar fish from these same lots were filleted and the fillets placed in frozen storage. Periodically during storage, measurements were made of some of the chemical changes that had taken place. This provided an opportunity, firstly to observe the seasonal variations in the EPN values of fish, and secondly to determine whether variations in the initial levels of extractable proteins were associated with any particular differences in the deterioration pattern when the fillets were frozen and stored.

Materials and Methods

The fish were "market" cod (*Gadus morhua*) that had been iced in trawlers from 24 to 96 hr before landing. They were taken from three fishing banks off the southeast coast of Nova Scotia; Browns, Emerald, and Western. A very small number of fish in some lots were in the late stages of rigor at the time of landing. These were not tested until rigor had been completely resolved. All others were filleted and tested immediately after they had been taken from the trawler. Other fillets from these same lots of fish were individually packaged in heat sealed, laminated plastic pouches, frozen in a plate freezer at –40 C and then stored at –3.3, –12, and –26 C. Frozen fillets were periodically removed from storage and tested. Each test of the stored fish was made on only one fillet. It was cut transversely into thin slices, and sections from the head, center, and tail areas were used to get a representative sample of the whole fillet.

Salt extractable protein determinations were carried out by the extraction procedure of Dyer et al. (1950, 1964). The colorimetric readings were made after the addition of Biuret solution (Snow 1950) and the results recorded as percent extractable protein nitrogen (EPN) in the muscle. The lipid content of the muscle was determined by the procedure of Bligh and Dyer (1959).

Results

The immediate postrigor EPN values throughout the year ranged between 1.81 and 2.39 with a mean of 2.13. Seventy-six percent of the fillets had values between 2.00 and 2.30; 10% were above 2.30, and 14% were below 2.00; none was above 2.38 or below 1.81.

Throughout most of the year, the mean monthly values were fairly constant between 2.17 and 2.21, but with a considerable spread for the individual values as shown in Fig. 1A. During March, April, and May there was a pronounced dip in the curve.

It is important to realize that the fish in March, April, and May did not all have low EPN values,

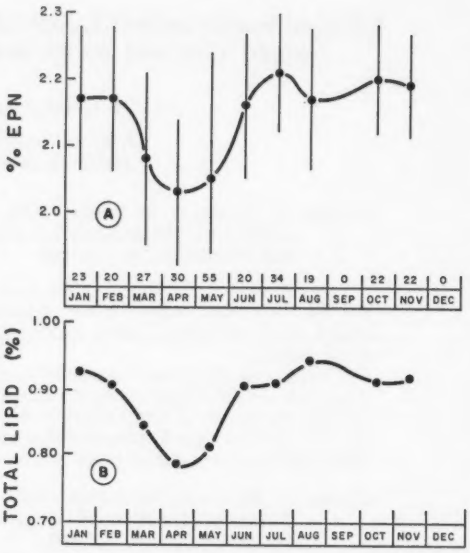

FIG. 1. A, Average monthly EPN values for cod fillets. Figures at the bottom represent the number of fish tested each month; the perpendicular lines indicate standard deviation from the means; B, Average monthly lipid content of cod fillets. Each point is the mean FFA value from tests on 10 fish.

and fish caught during the remainder of the year did not all have high values. This is illustrated by the vertical lines in Fig. 1A, showing the standard deviations from the monthly mean values.

The lipid content of the cod muscle also showed a slight decrease during March, April, and May, with minimum values coinciding with the minimum EPN content (Fig. 1B). This is similar to the results obtained previously for inshore cod by Dambergs (1964).

A relationship between the physiological condition of the fish (i.e. as affected by spawning and feeding) and the subsequent keeping time of the frozen fillets, as indicated by the insolubilization of proteins, appears evident (Fig. 2A and B). During frozen storage at both –3.3 C and –12 C, the EPN values of fillets from fish caught in the spring (March 1 and May 9) decreased more rapidly than those caught during the summer and fall (July, August, October, and November). For fillets that were frozen and stored at –26 C there was no measurable decrease in EPN values for storage periods up to 2 years and therefore no reflection of the season of catching on the rate of change (Fig. 3). It is interesting to observe, however, that slightly lower initial EPN values for

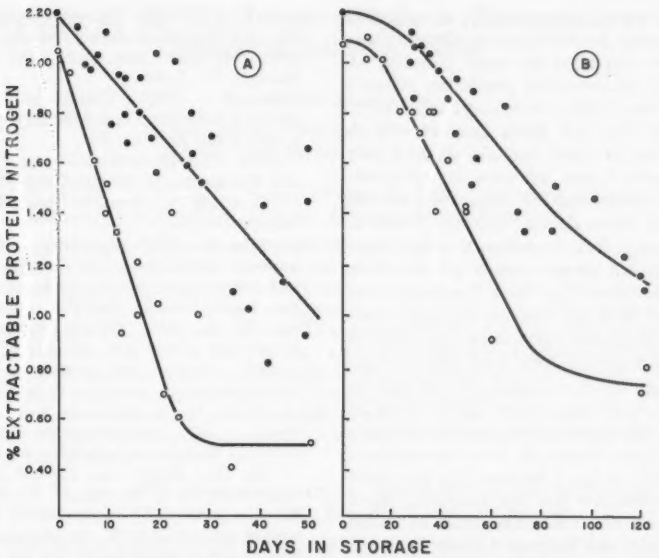

FIG. 2. A, EPN values for frozen cod fillets stored between 0 and 52 days at –3.3 C (26 F). The open circles are the values from fish caught during March and the solid circles are from fish caught during July, August, October, and November; B, The same as A except that the fillets were stored at –12 C (10 F).

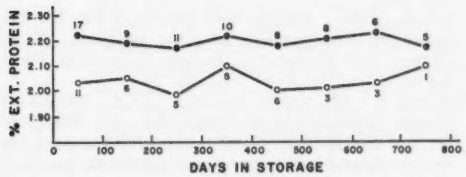

FIG. 3. EPN values of frozen cod fillets stored at –26.6 C (–16 F). Each point represents the average value of tests carried out during successive 100-day periods. The number of individual tests carried in each period is shown in the figures adjacent to the data points. The open circles are for fish caught in March and May and the solid circles are for fish caught in July, August, October, and November.

the fillets from the spring-caught fish persisted through the storage period when they were held at –26 C.

Discussion

The adverse effect of spawning on the chemical composition and general quality of fish muscle has long been recognized. The fishermen speak of the depleted fish after spawning as being "spent" and they are well aware that the muscle of these fish is much softer and more easily indented than that of normal fish. Dambergs (1964) found that during the spawning period fillets from Atlantic inshore cod had a decrease of approximately 20% in the fat content, 5% in the total proteins, and an increase in water solubles and total water content. Ironside and Love (1958) found a considerable variation in the salt-extractable protein of North Sea cod, some of which they associated with spawning and feeding cycles, as well as with the size and maturation of the fish. They even suggest that the absolute amounts of insoluble and soluble proteins furnish a sensitive guide to the nutritional status of the fish, once the normal variations have been established.

Our results from the tests with cod taken off the southeast coast of Nova Scotia are further confirmation of this line of thought. Prior to and during spawning there was a depletion in the nutritional condition of the fish, which in this instance was shown by decreases in the lipid and salt-extractable protein content of the muscle. With the North Sea cod, Ironside and Love (1958) suggested that slack winter feeding was also a cause of depletion, but this was not evident in the fish we examined.

When fillets from the depleted and normal fish were placed in frozen storage at –3.3 C and –12 C,

the former deteriorated more rapidly as indicated by the rate of protein insolubilization. But this was not observed when the same fish were stored up to 2 years at −26 C. As observed previously (Castell et al. 1968), there was little or no change in the EPN values for any of the cod fillets held at −26 C.

It must be borne in mind that the time of year when codfish spawn is not the same for all areas. The dates shown here were for fish caught off the southeast coast of Nova Scotia (Browns, Emerald, and Western Banks). But Canadian trawlers from the larger processing plants range all the way from Georges' Bank off the New England coast up to the Strait of Belle Isle and the southern coast of Labrador. As the trawlers go from south to north they encounter fish populations that spawn at increasingly later periods of the year. The results, in terms of months of the year when EPN values of fish from our western banks fall off, would not apply to fish from the Grand Banks or from the northern waters of the Gulf of St. Lawrence. In the more northern banks when the fish are spawning those from the banks off the southeast coast of Nova Scotia are well into the summer recovery period.

It should also be borne in mind that the condition of the fish examined, as indicated by the soluble protein content, showed considerable variation at all seasons of the year. It is very probable that with unselected fish from a commercial trawler the range would be even greater. This simply means that under natural conditions the individual fish do not spawn at exactly the same time; nor are they equally successful in their feeding efforts and they do not all feed upon the same type of food. But the general principle is true that during certain periods of the year, which will vary from one fishing bank to another, fish undergo these periods of depletion and recovery. For Atlantic cod taken from Browns, Emerald, and Western banks the maximum depletion for the majority of the fish occurred during March, April, and early May. During this period the average EPN values for mature cod drop about 7–10% when compared to fish at other periods of the year. It has been shown that fillets from these nutritionally depleted fish have a shorter life in frozen storage than those from normal fish, unless the storage temperature is reduced to the point where protein insolubilization is almost completely retarded (i.e. −25 C).

BLIGH, E. G., AND W. J. DYER. 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. 37: 911–917.

BYKOV, V. P. 1971. The solubility and aggregation of fish muscle proteins during freezing and refrigeration. Proc. All-Union Res. Inst. Mar. Fish. Oceanogr. Moscow 73: 7–35.

CONNELL, J. J. 1962. Changes in the amount of myosin extractable from cod flesh during storage at −14° C. J. Sci. Food Agr. 13: 607.

1969. Changes in the eating quality of frozen stored cod and associated chemical and physical changes, p. 323–338. *In* R. Kreuzer [ed.] Freezing and irradiation of fish.

DAMBERGS, N. 1964. Extractives of fish muscle. 4. Seasonal variations of fat, water solubles, protein and water in cod (*Gadus morhua* L.) fillets. J. Fish. Res. Board Can. 21: 703–709.

DYER, W. J. 1951. Protein denaturation in frozen stored fish. Food Res. 16: 522–527.

1967. Frozen fish muscle — chemical changes and organoleptic quality. Cryobiology 3: 297–305.

DYER, W. J., H. BROCKERHOFF, R. J. HOYLE, AND D. I. FRASER. 1964. Polyphosphate treatment of frozen cod. I. Protein extractability and lipid hydrolysis. J. Fish. Res. Board Can. 21: 101–106.

DYER, W. J., D. I. FRASER, D. G. ELLIS, AND W. A. MACCALLUM. 1957. Influence of intermittent short storage periods at 15°F., as encountered during refrigerator car transportation, on the quality of frozen cod stored at 0°F. J. Fish. Res. Board Can. 14: 627–635.

DYER, W. J., H. V. FRENCH, AND J. M. SNOW. 1950. Proteins in fish muscle. Extraction of protein fractions in fresh fish. J. Fish. Res. Board Can. 7: 585–593.

HEEN, E. 1954. Recent developments in fish freezing technique and pending scientific problems. Proc. Symp. Cured Frozen Fish Technol. Swed. Inst. Food Preserv. Res. SIK Publ. No. 100: 19 p.

IRONSIDE, J. I. M., AND R. M. LOVE. 1958. Studies on protein denaturation in frozen fish. I. Biological factors influencing the amounts of soluble and insoluble protein present in the muscle of the North Sea cod. J. Sci. Food Agr. 9: 597–604.

LOVE, R. M. 1962. Protein denaturation in frozen fish. VI. Cold storage studies on cod using the cell fragility method. J. Sci. Food Agr. 13: 269–278.

LOVE, R. M., AND I. ROBERTSON. 1967. Studies on the North Sea cod. IV. Effects of starvation, 2. Changes in the distribution of muscle protein fractions. J. Sci. Food Agr. 18: 217–220.

LUIJPEN, A. F. M. G. 1957. Denaturation of fish proteins. Nature 180: 1422–1423.

ROBERTSON, I., R. M. LOVE, AND W. P. COWIE. 1967. Studies on the North Sea cod. V. Effects of starvation, 3. Electrophoresis of the muscle myogens. J. Sci. Food Agr. 18: 563–565.

SNOW, J. M. 1950. Proteins in fish muscle. II. Colorimetric estimation of fish muscle protein. J. Fish. Res. Board Can. 7: 594–598.

Reproduction of the Bloodworm (*Glycera dibranchiata*) in the Sheepscot Estuary, Maine

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CREASER, E. P., JR. 1973. Reproduction of the bloodworm (*Glycera dibranchiata*) in the Sheepscot estuary, Maine. J. Fish. Res. Board Can. 30: 161–166.

The population of bloodworms (*Glycera dibranchiata*) at Wiscasset, Maine, is composed of five assumed year-classes. Most spawners are probably large 3- and 4-year-old bloodworms. At Wiscasset eggs are released into the coelom at a size of 21 μ. The rate of egg growth varies with season. In June 1–10 million eggs are spawned at a diameter of 151–160 μ. A bottom temperature in excess of 13 C seems necessary for spawning to occur. Spawning occurs in the afternoon, between 2 hr before and 1 hr after high water. Males emit streams of sperm while swimming at the surface; females swim rapidly at the surface and suddenly rupture, liberating all the eggs at once. Females are slightly more abundant than males in the flats. Spawning bloodworms are preyed upon by herring gulls (*Larus argentatus*) and striped bass (*Morone saxatilus*), while spent epitokes are consumed by the mud shrimp (*Crangon septemspinosus*), which in turn is eaten by striped bass.

CREASER, E. P., JR. 1973. Reproduction of the bloodworm (*Glycera dibranchiata*) in the Sheepscot estuary, Maine. J. Fish. Res. Board Can. 30: 161–166.

Nous touchons à plusieurs aspects de la reproduction de *Glycera dibranchiata*. L'analyse statistique des fréquences de longueur démontre que la population de Wiscasset, Maine, est composée de cinq présumées classes d'âge. La plupart des géniteurs sont probablement des grands vers de 3 ans ou des individus de 4 ans. Les oeufs de *G. dibranchiata* sont libérés dans le coelom. Ils mesurent alors 21 μ. Là, le rythme de croissance varie avec les saisons. La ponte de 1 à 10 millions d'oeufs, mesurant de 151 à 160 μ, a lieu en juin. D'après nos observations, la ponte ne commence que lorsque la température du fond dépasse 13 C. Elle a lieu l'après-midi, 2 heures avant et 1 heure après la marée haute. Le comportement de ponte des deux sexes diffère. Les mâles émettent des fils de sperme tout en nageant à la surface. Les femelles nagent rapidement à la surface et éclatent soudainement, libérant tous leurs oeufs à la fois. Les femelles sont légèrement plus abondantes que les mâles sur les plages, mais au moment de la ponte, on voit plus de mâles que de femelles. *G. dibranchiata* en frai est la proie du goéland argenté (*Larus argentatus*) et du bar d'Amérique (*Morone saxatilis*). Les épitoques vides servent de nourriture à la crevette de sable (*Crangon septemspinosus*).

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STUDIES on the bloodworm (*Glycera dibranchiata*) have been either physiologically or life history oriented. Recent investigations on *G. dibranchiata* have included studies of the hemoglobin (Vinogradov et al. 1970; Hoffmann and Mangum 1970; Seamonds et al. 1971a; Seamonds et al. 1971b; Li and Riggs 1971) and respiration (Mangum and Miyamoto 1970; Hoffman and Mangum 1972).

Although many aspects of the reproductive cycle of this species have already been reported in publications by Simpson (1962a, b) and Klawe and Dickie (1957), much remains unknown. This paper includes various aspects of the reproductive cycle that have either not been investigated or have been incompletely investigated by previous authors. These include spawning age and size, sex ratio, rate of gametogenesis, numbers of eggs laid, daily and seasonal triggering mechanisms, swarm density, sexual differences in spawning behavior, and predation.

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Materials and Methods

Most of the studies included in this paper were conducted between November 1967 and August 1969. The majority of the bloodworms used were collected from a small worm flat near Wiscasset, Maine, which was closed so that samples could be collected from an undisturbed population. Within this closed area, a section measuring 93 × 91.5 m was used for experimental purposes. A monthly sample was randomly chosen and dug from each of three positions designated high, middle, or low with respect to distance on the flat above the mean low-water mark. Each of the sample plots measured 30.5 × 1.5 m. Standard bloodworm hoes were used to collect samples.

All the bloodworms seen were removed and acclimated to high salinity water (approx 30–32‰) at the laboratory. Prior to immersion in anesthetic, gamete samples were withdrawn from the coelom of large bloodworms with the aid of capillary pipettes. Egg diameters and maximum oval sperm plate lengths were measured with an ocular micrometer and without a cover slip. Usually 10 eggs were measured from each worm. Bloodworms were anesthetized in 7.5% $MgCl_2$ by the method of Klawe and Dickie (1957) using a V-shaped measuring trough containing ample anesthetic to cover the worms. They were then revived in sea water before the sexes of all the worms were determined.

Female bloodworms used for egg counts were anesthetized, measured, and preserved. The worms were split lengthwise and washed thoroughly to remove as many eggs as possible. The body was then chopped into 1-cm pieces and these were immersed in sea water and stirred magnetically. After three or four changes of water, most of the remaining eggs were dislodged. The egg sample was diluted in as much as 9 liters of sea water. Two aliquots of 1 ml were withdrawn from this mixture, placed on Sedgwick–Rafter counting cells, and the numbers of eggs counted. The average figure was then multiplied by the dilution factor to obtain the final egg count.

During spawning, worms were captured underwater using scuba techniques and at the surface with dipnets from a boat.

The mean monthly water temperature was calculated from 25 thermister recordings collected in situ at 30-min intervals at hydrographic stations occupied for 12 hr. Mud temperature was determined in situ with a liquid filled temperature recorder or by digging a shallow hole in the mud flat and then inserting a thermometer horizontally at a depth of approximately 10 cm.

The polymodal frequency distribution analysis used in this paper conforms to the method of Harding (1949) as explained by Cassie (1950).

Results and Discussion

Length Frequency

The length frequency data in Fig. 1 give a somewhat misleading picture of the total number of various sized bloodworms in the mud flat as inefficiency in the capture of small individuals resulted in bias towards larger animals. It is evident, however, that, between June and July, nearly all the sexually mature bloodworms had left the mud and spawned. The majority spawned in June. One broken, spent epitoke was dug as late as August.

The February-June length frequency data were combined and analyzed statistically by Cassie's (1950) method (Table 1). Five modes were revealed and the percentages indicate that most of the bloodworms belonged to the assumed second and third year-class.

The assumption that these modes represent year-classes could be questioned for two reasons. Firstly, although the vast majority of bloodworms spawn during June, spawners were observed (rarely) during the winter months, and this results in the possibility of having two modes instead of one representing a year's data. Secondly, there is no known definite means of aging bloodworms.

The mean length, size range, number, and sex ratio of potential male and female spawners dug each month from the combined high, middle, and low positions are included in Table 2. There were no significant differences between these monthly male and female lengths ($t = .014$; $P < .05$, 8 df). Klawe and Dickie (1957) have recorded similar findings for a bloodworm sample dug at Yarmouth, Nova Scotia.

The mean lengths of potential male and female spawners fall between 32 and 36 cm. The modes of the third and fourth assumed year-classes (Table 1) fall at 27 and 36 cm. Therefore, most of the potential spawners are probably the larger 3-year-olds and the 4-year-old bloodworms.

Combined information from Fig. 1 and Table 1 shows that a small percentage of individuals in the assumed fourth year-class are nonspawners. This nonspawning fraction probably lives long enough to spawn as 5-year-olds. Five years, therefore, appears to be the maximum life span of the bloodworms at Wiscasset, Maine. Klawe and Dickie (1957) reached similar conclusions for bloodworms dug in Nova Scotia.

The size range of sexually mature bloodworms at Wiscasset was between 18 and 51 cm (Table 2). In Nova Scotia, Klawe and Dickie (1957) recorded the same as being between 13 and 36 cm. Although Simpson (1962a) did not designate the size range of sexually mature individuals obtained at Solomons, Maryland, all the specimens used in her studies were between 7 and 26 cm. Apparent geographic differences in the size of bloodworms may be attributed to the effects of temperature on growth and maturation (Gunter 1957) or possibly to differences in bloodworm races.

Rate of Gametogenesis

Sperm plates of approximately 14 μ oval length were first positively identified in October. By May 1, some measured 70–90 μ. By May 31, many of the sperm plates had broken down. Sperm plates within a given individual were usually similar in size but considerable variation occurred between individuals.

Eggs measuring 40–50 μ in diameter were first observed in the coelomic fluid during September (Fig. 2A). More recently I have observed that eggs are released from the ovaries sometime in August at a diameter of approximately 21 μ. The absence of eggs in the August sample probably indicates that ovulation occurred just after the August sampling

period. Klawe and Dickie (1957) reported that ovulation occurred in late August at a size of 20 μ. At Solomons, Maryland, this event occurred in mid-September at a diameter of 28 μ (Simpson 1962b).

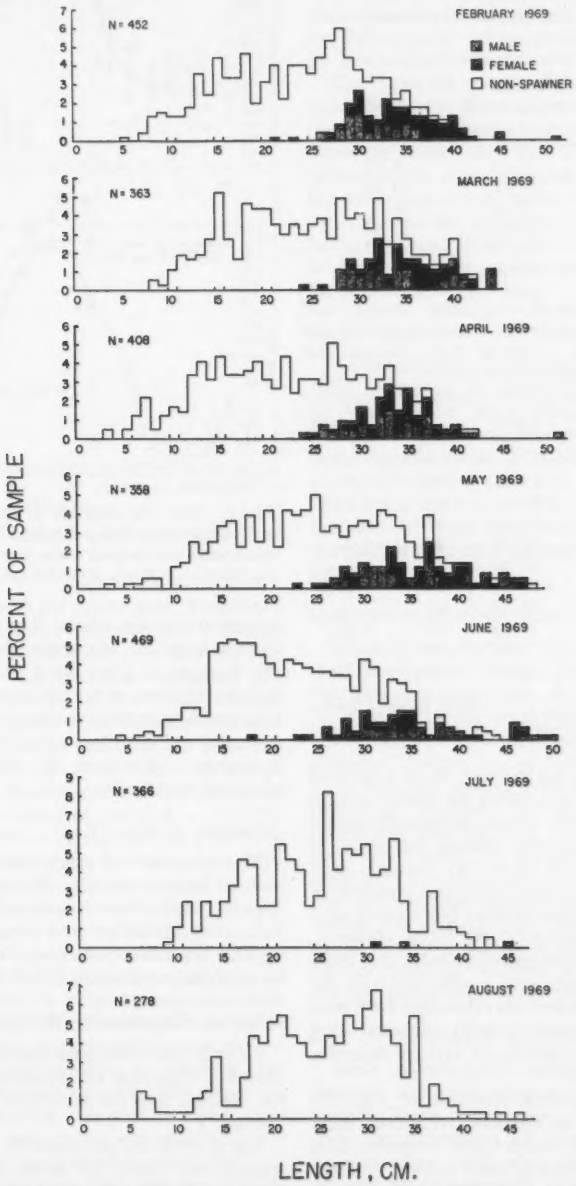

FIG. 1. The sexed length frequency distribution of combined high, middle, and low samples of bloodworms dug in the experimental area at Wiscasset.

TABLE 1. The calculated mean length, standard deviation, and percent of sample in each assumed year-class from the combined February–June polymodal frequency distribution analysis.

Assumed year class	Calculated mean length (*cm*)±1SD	% of sample
1	7±3	3.08
2	17±4	40.96
3	27±4	36.92
4	36±4	17.97
5	47±3	1.07

TABLE 2. The mean length, size range, number, and sex ratio of potential male and female spawners dug each month from the combined high, middle, and low positions in the experimental area.

Month (1969)	Mean length (*cm*)	Range (*cm*)	No.	Sex ratio (♀:♂)
Feb.	♀ 35	23–51	58	
	♂ 32	21–45	44	1.32:1
Mar.	♀ 35	24–44	44	
	♂ 34	28–44	43	1.02:1
Apr.	♀ 33	24–40	49	
	♂ 34	26–51	39	1.26:1
May	♀ 36	25–47	47	
	♂ 34	23–46	35	1.34:1
June	♀ 34	18–50	41	
	♂ 35	24–48	33	1.24:1
			Avg. ratio	1.24:1

Egg diameters increased to about 80–90 μ through to December–January but, during the coldest months of the year, egg growth almost completely ceased (Fig. 2A). Beginning in May, during the period of rising water temperature (Fig. 2B), the eggs grew rapidly and attained a mean spawning diameter of 151–160 μ by June. Nova Scotian bloodworm eggs were spawned at a diameter of 180–190 μ. However, Klawe (personal communication) revealed that these measurements were made with a cover slip over the eggs and the actual diameter may therefore have been slightly smaller. Simpson (1962b) recorded that bloodworm eggs were shed at an average diameter of 140 μ at Solomons.

The egg growth pattern recorded in Fig. 2A strongly suggests that, at Wiscasset, *G. dibranchiata* spawns annually. It is noteworthy, however, that during the course of several years a total of three bloodworms in spawning condition have been collected during the winter months. This small percentage of winter spawners is insignificant when

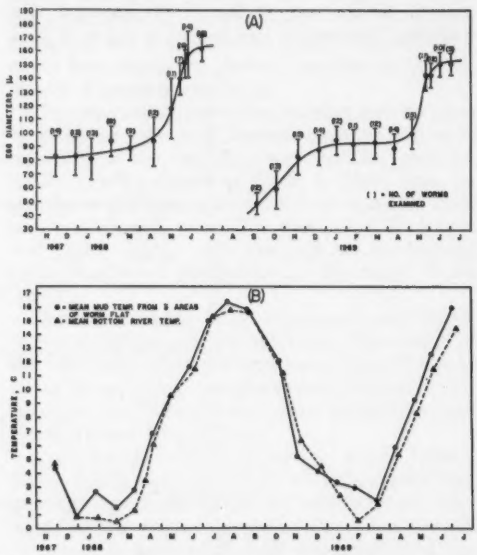

FIG. 2. (A) The monthly growth of bloodworm eggs with the coelom (lines fitted by eye); (B) The monthly mean mud and bottom water temperature recorded from the Sheepscot River at Wiscasset.

compared to the whole. The annual spawning of bloodworms at Wiscasset therefore agrees with the findings of Klawe and Dickie (1957) in Nova Scotia. However, they state that this annual spawning occurred in mid-May, whereas at Wiscasset spawning occurred in June. Simpson (1962a) reported that at Solomons, Maryland, *G. dibranchiata* probably spawned biannually.

NUMBERS OF EGGS LAID

The numbers of eggs laid by bloodworms of various lengths are recorded in Fig. 3. Klawe and Dickie (1957) have calculated that a bloodworm measuring 22–24 cm may contain 1.5–2.0 million eggs. A Wiscasset bloodworm of this length would be expected to contain 3.0–3.5 million eggs.

SEASONAL TRIGGERING MECHANISM

At least two biological factors are related to the seasonal triggering of spawning: the formation of the epitoke, and the growth of eggs to a spawning "range."

Egg growth did not cease for an extended period immediately prior to being spawned (Fig. 2A). During this time, it appears that the eggs were still increasing in diameter but at a decreasing rate. It is also apparent from Fig. 2A that the egg diame-

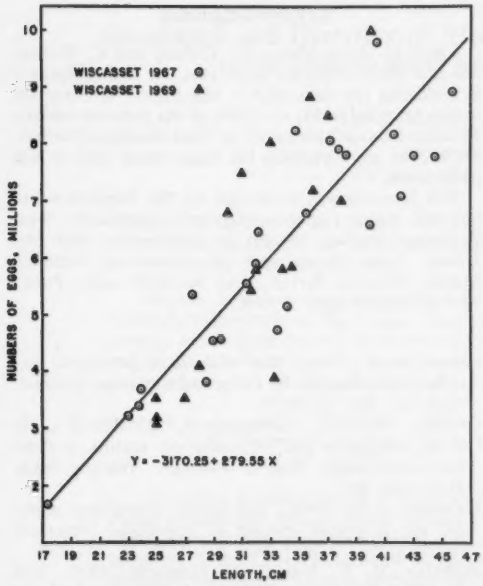

FIG. 3. The number of eggs produced as a function of bloodworm length.

ters at spawning varied from year to year. A student's t test was used to compare diameters of 140 eggs from the 14 worms collected just prior to spawning and 158 eggs from the 18 worms collected just after the onset of spawning in 1968 with 100 eggs from 10 worms collected prior to spawning and 50 eggs from the 5 worms collected just after the onset of spawning in 1969. A significant difference existed between the diameters of the two groups of eggs spawned on consecutive years ($t = 7.3210$; $P < .05$; 446 df). This suggests that the growth of eggs to a definite spawning size is not the most critical factor in determining when spawning occurs. However, egg growth to a certain diameter "range" definitely is related to the act of spawning.

A definite mud or bottom water temperature may initiate spawning. During the period April 22–June 23, 1969, bottom water temperatures were continuously measured with a temperature recorder. Using this information together with visual spawning observations, it was possible to conclude that spawning first occurred on June 18, 1969, when the bottom water temperature was slightle higher than 13 C and the mud temperature was approximately 14 C. Similar bottom water temperatures have been noted during spawnings since 1969. Simpson (1962a) found little correlation between absolute temperature and the suggested breeding periods, except that temperature is approaching its maximum during June and July, and its minimum during November and December. She recorded the occurrence of spawning in the fall at Solomons during years when the falling water temperatures were in the range of 10.3–5.5 C (1944) and 18.7–13.8 C (1960).

The apparent direct effect of temperature on spawning in the Wiscasset area is also revealed by the fact that spawning occurred only in the afternoon on those days during which the sun was shining on the exposed mud flats during the previous low tide. Apparently, when the water temperature was close to the spawning point, exposure of the flat to the heat of the sun at low water heated the mud so that the incoming tide was warmed above the spawning temperature.

Daily Triggering Mechanism

An important environmental factor related to the daily triggering of spawning is tidal amplitude. At Wiscasset, a mean tidal amplitude of 2.9 m made it very easy to recognize the fact that spawning began approximately 2 hr before high water, continued during high tide, and, sometimes, continued for as much as 1 hr after high water. Simpson (1962a) has also recorded that bloodworms at Solomons spawned around the high water of the "second daily tide."

Emergence from the Flat

Scuba observations on bloodworms have revealed that bloodworms emerge from the mud anterior end first and then swim vertically to the surface. In addition to the characteristic lateral swimming undulations of spawning individuals described by Simpson (1962a), the worms sometimes appeared to be rotating slowly. Both surface and scuba observations revealed that swarming worms were found only over the flats; none were observed over the river channel. At the surface, the bloodworms leveled off on a more horizontal plane, but the anterior portions were slightly higher than the posterior region. Occasionally, the anterior portions broke the surface. The density of bloodworms was similar to the densities recorded at Solomons (Simpson 1962a), about one worm per 36-m^2 surface area.

Sexual Differences in Spawning Behavior

Male bloodworms usually emitted two long streams of sperm while swimming slowly at the surface. These streams were very close together and gave the impression of being entwined with one another. They were also apparently cohesive and did not dissipate rapidly (they were evident for up to 1 m behind the spawning worm). On June 16, 1967, the duration of sperm emission was deter-

mined for three worms as being 2, 5, and 4.5 min. After this short period at the surface, they descended. Female bloodworms, on the other hand, swam very rapidly at the surface for no longer than a minute; then, suddenly, the body wall ruptured near the posterior one-third of the worm, and a cloud of eggs was liberated. The females then slumped and, wiggling feebly, sank to the bottom.

Both Simpson (1962a) and Klawe and Dickie (1957) reported that gametes were released from the posterior end. In addition, Klawe and Dickie (1957) observed that "On several occasions, worms discharged their gametes through the posterior end, the body wall, or the proboscis without special inducement."

The emission of gametes was not confined solely to the surface waters. On one occasion, using scuba gear in 3 m of water, I observed a male bloodworm swimming in a vertical position just above the bottom. Its tail was scarcely 5–8 cm from the mud. After swimming in this position for several minutes, emitting steady streams of sperm, and being swept by the tide for perhaps 20 m, the worm proceeded to lie horizontally on the mud and burrowed in. No positive indication of female bloodworms spawning below the surface has yet been observed by the author.

Some male bloodworms may spawn on more than one occasion. Using scuba gear, I have observed some male bloodworms digging back into the mud. However, the fact that partially spawned males were only rarely dug from the mud flats leads me to believe that spawning during more than one high tide may be exceptional. All females observed on the bottom were "spent"; none were observed burrowing back into the mud.

My observations coincide with Klawe and Dickie's (1957) belief that all bloodworms die after spawning. Differences in the degree of atrophy of the gut at swarming led Simpson (1962a) to believe that some spawners could survive shedding.

Predation

An increasing number of herring gulls (*Larus argentatus*) circling and diving in the area of Wiscasset bloodworm flats on the incoming tide as it approached high water in June was almost a sure indication that *G. dibranchiata* was spawning. All the stomachs of striped bass (*Morone saxatilis*) captured during bloodworm spawning periods contained epitokes. Simpson (1962a) also reported gulls and striped bass feeding upon bloodworms.

Many bass stomachs also contained quantities of the mud shrimp (*Crangon septemspinosus*), which was observed to consume spent epitokes that lay on the bottom.

Acknowledgments

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Cassie, R. M. 1950. The analysis of polymodal frequency distributions by the probability paper method. N.Z. Sci. Rev. 8: 89–91.

Gunter, G. 1957. Temperature, p. 159–184. *In* J. E. Hedgpeth [ed.] Treatise on marine ecology and paleocology. Vol. 1. Ecology. Waverly Press, Baltimore, Md.

Harding, J. P. 1949. The use of probability paper for the graphical analysis of polymodal frequency distributions. J. Mar. Biol. Ass. U.K. 28: 141–153.

Hoffman, R. J., and C. P. Mangum. 1970. The function of coelomic cell hemoglobin in one polychaete *Glycera dibranchiata*. Comp. Biochem. Physiol. 36: 211–228.

1972. Passive ventilation in benthic annelids? Science 176: 1356.

Klawe, W. L., and L. M. Dickie. 1957. Biology of the bloodworm, *Glycera dibranchiata* Ehlers, and its relation to the bloodworm fishery of the Maritime Provinces. Fish. Res. Board Can. Bull. 115: 37 p.

Li, S. L., and A. F. Riggs. 1971. Partial sequence of the NH_2 — terminal segment of *Glycera* hemoglobin: homology with sperm whale myoglobin. Biochim. Biophy. Acta 236: 208–210.

Mangum, C. P., and D. M. Miyamoto. 1970. The relation between spontaneous activity cycles and diurnal rhythms of metabolism in the polychaetous annelid *Glycera dibranchiata*. Mar. Biol. (N.Y.) 7: 7–10.

Seamonds, B., R. E. Forster, and A. J. Gottlieb. 1971a. Heterogeneity of the hemoglobin from the common bloodworm *Glycera dibranchiata*. J. Biol. Chem. 246: 1700–1705.

Seamonds, B., R. E. Forster, and P. George. 1971b. Physiochemical properties of the hemoglobin from the common bloodworm *Glycera dibranchiata*. J. Biol. Chem. 246: 5391–5397.

Simpson, M. 1962a. Reproduction of the polychaete *Glycera dibranchiata* at Solomons, Maryland. Biol. Bull. (Woods Hole) 123: 396–411.

1962b. Gametogenesis and early development of the polychaete *Glycera dibranchiata*. Biol. Bull. (Woods Hole) 123: 412–423.

Vinogradov, S. N., C. A. Machlik, and L. L. Chas. 1970. The intracellular hemoglobins of a polychaete. Some properties of the hemoglobins of *Glycera dibranchiata*. J. Biol. Chem. 245: 6528–6533.

Abundance and Diversity of Mollusca in an Industrialized Portion of the Ottawa River near Ottawa–Hull, Canada

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MACKIE, G. L., AND S. U. QADRI. 1973. Abundance and diversity of Mollusca in an industrialized portion of the Ottawa River near Ottawa–Hull, Canada. J. Fish. Res. Board Can. 30: 167–172.

Organic pollution was a major factor in limiting the abundance and diversity of Gastropoda and Pelecypoda in the Ottawa River near Ottawa–Hull. Analyses of 472 Ekman grab samples taken from May to November of 1968 showed that the average standing crops (no./m²) of mollusks were larger in areas immediately upstream than in areas immediately downstream of slaughter house and pulp and paper mill outfalls.

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La pollution organique est le principal facteur limitant la répartition des gastéropodes et des pélécypodes dans la rivière Outaouais, près d'Ottawa–Hull. L'analyse de 472 échantillons recueillis avec une benne d'Ekman de mai à novembre 1968 démontre que la biomasse moyenne (no./m²) de mollusques est significativement plus grande dans les régions directement en amont d'effluents d'abattoir et d'usine de pâte et de papier que dans les régions situées directement en aval.

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THIS study was planned to compare the abundance and diversity of Gastropoda and Pelecypoda during May–November 1968 in portions of the Ottawa River near Ottawa–Hull immediately upstream and downstream of outfalls from a slaughter house and two pulp and paper mills. The only major studies to have been carried out on the Ottawa River prior to 1968 were water quality surveys (Thomas 1952; Piché 1954; Barrens 1961; LeSauteur 1965) and a growth study on perch (Stobo 1971).

Study Area and Materials and Methods

The study area, 26 km long and situated between Britannia Bay and the Township of Gatineau (45°22′–45°24′N 75°53′–75°35′W), was divided into five stations; the locations and some characteristics of these are given in Fig. 1 and Table 1. Station 1, being relatively clean, was chosen as a control over Station 2, which carried organic wastes from a slaughter house, a pulp and paper mill, and several raw sewage outfalls, and had higher biochemical oxygen demand (BOD), turbidity, and coliform bacteria counts than Station 1 (Table 2). Station 3 was chosen as a control over Stations 4G and 4K, which were located below a pulp and paper mill. The water currents were such that most of the pulp and paper wastes were restricted to the Gatineau shore (Station 4G) with very little moving toward the Kettle Island shore (Station 4K). Consequently, a higher percentage of organic matter in the sediments (Table 1) and a higher average BOD and water turbidity (Table 2) were present in Station 4G than in Station 4K.

The following determinations were made weekly on both bottom and surface waters. Velocities were computed from discharge and stage data supplied by the

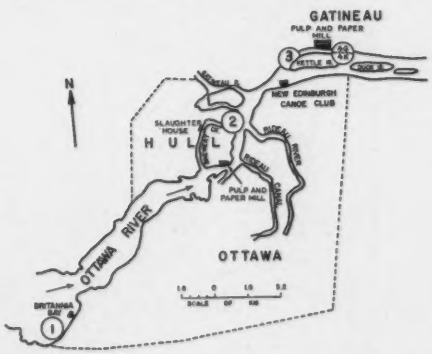

FIG. 1. Map of the study area and locations of the stations.

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TABLE 1. Some characteristics of five stations studied in the Ottawa River. Frequency of aquatic plant species given as percent occurrence in Ekman grab samples (average 88) collected at 0–2-m depths.

	Station and morphometry	Substrate type	Frequency of submerged aquatic plants (%)		Pollutants
1	Control Britannia Bay–(Ontario shore) Area = 2.4 km² Mean depth = 1.7 m Shore slope = 0.3 m/15 m Water velocity = 0.1–0.4 m/sec.	Very fine sand (0.0625–0.125 mm). % volatile matter = 1.0	*Potamogeton* spp. *Elodea canadensis* *Myriophyllum* sp. *Vallisneria americana*	70 3 2 1	Relatively clean with few industrial effluents.
2	Slaughterhouse and pulp and paper mill (below outfalls)–Brewery Creek Area = 0.2 km² Mean depth = 2.0 m Shore slope = 1 m/15 m Water velocity = 0.1–2.1 m/sec	Fine sand (0.125–0.250 mm) overlain with a thick layer of plant and animal detritus. % volatile matter = 20.9	*E. canadensis* *Myriophyllum heterophyllum* *M. alterniflorum* *V. americana*	46 2 2 2	Organic pollution from slaughter house, pulp and paper mill, and untreated sewage from Hull.
3	Control–pulp and paper mill (above outfalls)–Kettle Island Area = 0.3 km² Mean depth = 3.5 m Shore slope = 2.5 m/15 m Water velocity = 0.6–3.8 m/sec	Fine sand with little plant debris. % volatile matter = 4.6	*V. americana* *E. canadensis*	25 1	Diluted sewage from Hull and Gatineau Pointe. Diluted effluents from slaughter house.
4G	Pulp and paper mill (Below outfalls). Gatineau shores. Area = 0.6 km² Mean depth = 3.2 m Shore slope = 1.5 m/15 m Water velocity = 0.6–3.8 m/sec	Thick layer of wood fibers. % volatile matter = 41.1	No plants		Wood fibers and chemicals from pulp and paper mill. Untreated sewage from Gatineau Township.
4K	(Below and across from outfalls) Kettle Island. Area = 0.3 km² Mean depth = 3.2 m Shore slope = 1.5 m/15 m Water velocity = 0.6–3.8 m/sec.	Fine sand with thick layer of woodchips and some wood fibers. % volatile matter = 22.3	*V. americana*	2	Dilution of pollutants from Station 4G.

TABLE 2. Means and 95% confidence intervals of some physical, chemical, and bacterial determinations of the water at each station. Numbers of samples in parentheses. All values are averages for 1968 and 1969 and are in ppm unless otherwise shown.

	Stations					
	1	2	3	4G	4K	Pollution Criteria (Ontario Water Resources Commission 1970)
*p*H	7.2±0.1 (36)	6.9±0.1 (38)	6.9±0.1 (36)	6.8±0.1 (20)	6.8±0.1 (16)	>6.5, <8.5
Dissolved oxygen	9.4±0.7 (37)	8.2±0.4 (38)	8.0±0.5 (33)	7.7±0.6 (21)	8.4±0.8 (14)	>5
Carbon dioxide	9.4±0.7 (35)	11.9±1.0 (38)	10.2±0.6 (33)	12.7±2.0 (21)	10.3±0.5 (14)	<25
Conductivity	68.3±5.0 (21)	77.2±3.8 (24)	64.3±4.0 (20)	54.4±13.0 (14)	59.0±11.0 (6)	No increase > 1/3 of natural state
Alkalinity	25.8±2.0 (16)	26.5±1.6 (16)	24.1±2.2 (17)	24.1±1.0 (11)	23.5±4.4 (6)	<20
BOD	1.1±0.5 (12)	3.8±2.4 (12)	1.6±0.6 (13)	6.8±8.4 (8)	1.2±1.2 (5)	<4
Calcium	10.3±0.8 (8)	10.8±0.5 (8)	9.8±0.4 (9)	10.0±0.6 (5)	10.0±0 (4)	No criterion given
Hardness (Ca)	24.9±2.3 (8)	28.1±2.9 (10)	27.7±0.4 (9)	38.5±10.2 (8)	20.0±0 (2)	No criterion given
Nitrate nitrogen	0.06±0.04 (8)	0.11±0.08 (8)	0.09±0.07 (9)	0.10±0.11 (5)	0.06±0.03 (4)	Should not cause nuisance plant growth
Phenols ppb	5.6±3.6 (8)	6.0±2.1 (10)	5.6±2.9 (7)	9.2±1.3 (5)	10.5±11.8 (4)	Should not taint fish
Orthophosphate	0.12±0.04 (16)	0.60±0.45 (16)	0.14±0.03 (17)	0.14±0.07 (11)	0.16±0.09 (6)	Should not cause nuisance plant growth
Sulfate	15.0±1.1 (16)	15.5±2.2 (12)	15.1±1.5 (11)	21.8±6.7 (9)	14.0±10.5 (2)	
Turbidity JTU[a]	17.4±4.1 (16)	24.7±2.0 (18)	18.6±5.5 (17)	45.5±34.9 (11)	21.0±4.3 (6)	No artificial increase > 25 JTU
Total coliforms M.P.N.[b]/100 ml	760±935 (21)	322200±1014292 (18)	8388±2988 (17)	342042[c]±277645 (22)		<1000/100 ml
Fecal coliforms M.P.N./100 ml	131±194 (21)	68410±57819 (18)	2090±1023 (17)	130485±38808 (22)		<100/100 ml

[a]Jackson turbidity units.
[b]Most Probable Number.
[c]Values at Stations 4G and 4K are averaged together for total coliform and fecal coliform bacteria.

TABLE 3. Mollusca species composition (average numbers/m² for six samples every 2 weeks) at each station during May–November 1968. (Species are ranked from those found only at Station 1 to those found everywhere but at Station 1.)

Species	Stations				
	1	2	3	4G	4K
Gastropoda					
Goniobasis livescens	3.8	0	0	0	0
Helisoma trivolvis	1.3	0	0	0	0
Helisoma campanulata	–[a]	0	0	0	0
Pomatiopsis lapidaria	15.8	0	0	0	0
Lymnaea catascopium	60.4	0	0.4	0	0
Amnicola deceptans	23.1	–[a]	0.4	0	0
Probythinella lacustris	–[a]	–[a]	0	0	0
Helisoma anceps	4.1	1.9	0	0	–[a]
Valvata sincera	1.9	2.3	–[a]	0	0
Gyraulus hirsutus	2.5	0.1	2.3	0	2.3
Gyraulus deflectus	3.8	0.4	–[a]	0	2.2
Promenetus exacuous	1.8	8.3	–[a]	0	–[a]
Gyraulus parvus	13.6	6.7	60.6	0	0
Valvata tricarinata	68.1	2.5	–[a]	0	0
Amnicola limosa	105.3	11.2	10.8	0	25.0
Physa gyrina	3.8	2.8	17.7	–[a]	3.6
Ferrissia parallela	–[a]	–[a]	3.9	0	0
Campeloma decisum	6.4	2.5	89.4	29.9	44.1
Bithinia tentaculata	0	1.2	8.2	0	4.5
Total Gastropoda	315.7	39.5	193.7	29.9	81.7
Pelecypoda					
Sphaerium striatinum	86.5	0	0	0	0
Pisidium walkeri	0.7	0	0	0	0
Pisidium fallax	0.3	0	0	0	0
Sphaerium securis	16.3	0	2.0	0	–[a]
Sphaerium transversum	3.9	0	1.2	0	0
Pisidium dubium	3.6	0	–[a]	0	0
Pisidium lilljeborgi	83.4	0	0.3	0.9	3.5
Pisidium casertanum	72.6	2.6	91.0	11.6	97.0
Pisidium compressum	7.9	–[a]	1.5	0	2.1
Pisidium nitidum	32.9	2.1	2.0	3.1	0.4
Pisidium ferrugineum	3.3	0	1.4	0	–[a]
Elliptio complanatus	10.3	0	4.5	–[a]	2.1
Lampsilis radiata	0.7	0	4.0	0	0
Sphaerium lacustre	0.3	1.5	0	0	0
Sphaerium partumeium	0.7	1.6	–[a]	0	0
Pisidium amnicum	0.3	0	0.3	0	0.4
Pisidium adamsi	3.7	0	0.3	1.7	0.4
Pisidium punctatum	0	0	3.8	0	0.4
Pisidium supinum	0	0	0.9	0	–[a]
Pisidium aequilaterale	0	0	0.9	0	–[a]
Sphaerium corneum	0	0	1.0	0	0
Sphaerium occidentale	0	0	0.1	0	0
Total Pelecypoda	327.4	7.8	115.2	17.3	106.3

[a]Species were found in samples taken in 1969 but not in 1968.

Canada Department of Environment. Dissolved oxygen, dissolved carbon dioxide, and *p*H were determined with a "Hach Water Chemistry Field Kit." Conductivity was measured with a portable "Beckman Conductivity Meter."

Every 2 weeks during the study period, we sent water samples to the Ontario Water Resources Commission, which analyzed bottom and surface waters for 15 characters, including BOD, orthophosphate, nitrate, nitrite, and calcium hardness. These characters were also examined in 1969 (May-November) but we used a "Direct Reading E. L. Hach Chemical Kit" with its Instruction Manual (4th ed.) to analyze them; the results are included in this report only to increase the sample size for calculating a 95% confidence interval about the mean of each chemical and physical characteristic (Table 2). The Ontario Department of Public Health examined bottom and surface waters for Most Probable Numbers (M.P.N.) of total coliform and total fecal coliform bacteria.

The gastropods and pelecypods were sampled using a random sampling technique so that as many different sites as possible were sampled at each station. An average of six samples was collected from a different site every 2 weeks at each station yielding a total of 472 Ekman grab samples in 1968. Some mollusk species were found in a 1969 study but not in the 1968 study. To give a more accurate picture of the mollusk diversity at each station, the species found in 1969 are also reported but, since a different sampling technique was used, no numerical values of abundance are given (see Table 3).

A 15 × 15-cm standard Ekman grab, with a screen on top, was used to take the bottom samples. The Ekman grab was shown to be more efficient than other grabs in soft sediments (Flannagan 1970). Usually six samples of the sediments were taken and combined for washing through a nest of two sieves, the smallest mesh having 0.37-mm openings (Mackie and Qadri 1971a).

The gastropods and pelecypods were hand sorted from the debris and preserved in 70% ethanol. Taxonomic keys by Pennak (1953) and Berry (1943) were used to identify the gastropods, and keys by Herrington (1962) and Clarke and Berg (1959) to identify the pelecypods. Results are reported as numbers per square meter.

Percentage estimates of organic matter in the sediments were made by ashing and weighing three samples from each station. All animals were removed from the samples before ashing. The results are expressed as percent volatile matter.

Student's *t* test for small samples (Simpson et al. 1960, p. 176) was used to determine significant differences in average standing crops (no./m^2) of gastropods and pelecypods between stations. Differences are considered significant at $P \leq 0.05$.

Difference Between Upstream and Downstream Stations

Higher densities ($P \leq 0.05$) and a greater diversity of gastropods were present at Station 1 than at Station 2 (Table 3). The predominant group of gastropods at Station 2 were those that are associated with aquatic vegetation; such species as *Amnicola limosa*, *Physa gyrina*, *Promenetus exacuous*, and *Gyraulus* spp. were more abundant on the plants *Myriophyllum* spp., *Elodea canadensis*, and *Vallisneria americana* than in the sediment below the plants. Similar results were obtained by Mackie and Qadri (1971b) and Mackie (1971). Densities of mollusks that are typically associated with the sediments, i.e. *Campeloma decisum* and all pelecypods, were significantly ($P < 0.05$) lower at Station 2 than at Station 1.

There were also higher densities ($P \leq 0.05$) and greater diversities of gastropods and pelecypods at Station 3 than at Stations 4G and 4K (Table 3). The absence of aquatic plants at Station 4G was accompanied by the absence of plant associated mollusks. The only mollusks present at Station 4G were those typically associated with the sediments but they were found only in the deeper waters fringing on Station 4K. Although aquatic vegetation was present at Station 4K, plants were so infrequent in grab samples that densities of all gastropods associated with them were significantly lower than at Station 3. Consequently, mollusks, such as *C. decisum* and *Pisidum casertanum*, that are associated, with the sediments were more abundant than those living on aquatic vegetation i.e. *Gyraulus* spp., *A. limosa*, and *P. gyrina*.

The low densities of most gastropods at Stations 2, 4G, and 4K can be directly or indirectly related to organic pollution. Suspensions of organic wastes at these stations probably imposed large BOD or had a smothering effect on both the plants and the gastropods. The slaughter house and raw sewage wastes at Station 2 and wood fibers at Stations 4G and 4K eventually settled to the bottom and covered the fine sandy sediments, which otherwise supported large mollusk populations as in Stations 1 and 3. Most chemical factors (Table 2) probably had little effect on mollusk populations since the magnitudes of these factors were well below the criteria recommended by the Ontario Water Resources Commission (1970). This does not exclude the possible synergistic effects that some chemicals may have on mollusks, but this aspect was not tested.

Acknowledgments

The gastropods and unionids were identified or verified by Dr A. H. Clarke, Jr., of the National Museum of Canada. Reverend H. B. Herrington identified the sphaeriids. Our thanks are also extended to Dr Clarke for his criticisms of the manuscript. Water samples were analyzed for water chemistry by the Ontario Water Resources Commission and for coliform bacteria by the Ontario

Department of Public Health. This research was supported by N. R. C. grant No. A-2386 and the Canada Department of the Environment, Ottawa, grant HIK.

BARRENS, R. 1961. Report on water pollution survey of the Ottawa River from Pointe Fortune to Timiskaming. Ont. Water Res. Comm., Toronto, Ont. 37 p. 1 map.

BERRY, E. G. 1943. The Amnicolidae of Michigan: distribution, ecology, and taxonomy. Misc. Publ. Mus. Zool. Univ. Mich. 57: 68 p.

CLARKE, A. H., JR., AND C. O. BERG. 1959. The freshwater mussels of central New York. Cornell Univ. Agr. Exp. Sta. Mem. 367: 79 p.

FLANNAGAN, J. F. 1970. Efficiencies of various grabs and corers in sampling freshwater benthos. J. Fish. Res. Board Can. 27: 1691–1700.

HERRINGTON, H. B. 1962. A revision of the Sphaeriidae of North America (Mollusca: Pelecypoda). Misc. Publ. Mus. Zool. Univ. Mich. 118: 74 p. 7 pl.

LESAUTEUR, T. 1965. Rapport sur l'état des eaux de la Rivière Outaouais (1965). Que. Water Comm., Quebec, Que. 55 p.

MACKIE, G. L. 1971. Some aspects of the distribution and ecology of macrobenthos in an industrialized portion of the Ottawa River near Ottawa and Hull, Canada. M.Sc. Thesis, Univ. Ottawa, Ottawa, Ont. 161 p.

MACKIE, G. L., AND S. U. QADRI. 1971a. A new sieve and tub-trough for washing substrate samples aboard a boat. Progr. Fish-Cult. 33: 112–115.

1971b. A quantitative sampler for aquatic phytomacrofauna. J. Fish. Res. Board Can. 28: 1322–1324.

ONTARIO WATER RESOURCES COMMISSION. 1970. Guidelines and criteria for water quality management in Ontario. Ont. Water Res. Comm., Toronto, Ont. 26 p.

PENNAK, R. W. 1953. Fresh-water invertebrates of the United States. Ronald Press, New York, N.Y. 769 p.

PICHÉ, L. 1954. Report on the pollution of the Ottawa River and its principal tributaries — between Ottawa–Hull and the island of Montréal in 1954. Anti-pollution League of Quebec, Que. Wildl. Fed., Quebec, Que. 62 p.

SIMPSON, G. G., A. ROE, AND R. C. LEWARTIN. 1960. Quantitative zoology. Harcourt, Brace and Co., New York, N.Y. 400 p.

STOBO, W. 1971. Distribution and growth of yellow perch in the polluted and unpolluted waters of the Ottawa River. M.Sc. Thesis, Univ. Ottawa, Ottawa, Ont. 124 p.

THOMAS, T. F. J. 1952. Ottawa River drainage basin, 1947–48. Water survey report no. 2. Dep. Mines Tech. Surv. Can., Mines Br. Rep. 834: 114 p.

Underwater Observations on Tracks of Dredges and Trawls and Some Effects of Dredging on a Scallop Ground

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CADDY, J. F. 1973. Underwater observations on tracks of dredges and trawls and some effects of dredging on a scallop ground. J. Fish. Res. Board Can. 30: 173–180.

Tracks of three types of fishing gear in bottom sediments were observed from a submersible in Chaleur Bay (Gulf of St. Lawrence). Tracks left by past otter trawling activities covered at least 3% of the bottom by area and were considered to have been made by trawl doors.

Shallow tracks made by inshore and offshore scallop dredges during the course of the study could be distinguished from each other and from trawl tracks.

Scallop dredging lifts fine sediments into suspension, buries gravel below the sand surface, and overturns large rocks embedded in the sediment, appreciably roughening the bottom. The inshore Alberton dredge is inefficient, dumping its contents back onto bottom at intervals during the tow.

Dredging causes appreciable lethal and sublethal damage to scallops left in the track, this damage being greatest on rough bottom. Incidental mortalities to scallops with an offshore dredge of at least 13–17% per tow are of the same order of magnitude as estimates of harvesting efficiency made in earlier studies.

Predatory fish and crabs were attracted to the dredge tracks within 1 hr of fishing and were observed in the tracks at densities 3–30 times those observed outside the tracks.

CADDY, J. F. 1973. Underwater observations on tracks of dredges and trawls and some effects of dredging on a scallop ground. J. Fish. Res. Board Can. 30: 173–180.

L'auteur a observé d'un sous-marin les sillons tracés sur les sédiments du fond par trois types d'engins de pêche dans la baie des Chaleurs (golfe Saint-Laurent). Les sillons laissés par les opérations passées de chalutage couvrent au moins 3% de la superficie du fond, et sont considérés comme ayant été tracés par les panneaux de chalut.

Les sillons peu profonds laissés par les dragues à pétoncles côtières et hauturières sont reconnaissables les uns des autres et diffèrent des traces de chalut.

Le draguage des pétoncles soulève et laisse en suspension les sédiments fins, enterre le gravier sous la surface de sable et retourne les grosses roches enfouies dans le sédiment, contribuant de façon appréciable à rendre les fonds rugueux. La drague côtière d'Alberton est inefficace, puisqu'elle déverse périodiquement son contenu sur le fond pendant la traînée.

Le draguage a des effets létaux et sublétaux appréciables sur les pétoncles laissées dans les sillons. Le maximum de dommage se produit sur fonds rugueux. Les mortalités incidentelles causées aux pétoncles par la drague hauturière, estimées à au moins 13–17% par coup de drague, sont du même ordre que les estimations d'efficacité de récolte faites au cours d'études antérieures.

Les poissons prédateurs et les crabes sont attirés vers les sillons en moins d'une heure. On les a vus dans les sillons à des densités de trois à 30 fois supérieures à celles d'en dehors des sillons.

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BEFORE the development of modern techniques of underwater photography and manned submergence, it was difficult or impossible to assess incidental effects of dredging or trawling on the sea floor and earlier investigators (e.g. Graham 1955) concluded that there were no significant differences between the benthos of fished and unfished grounds. More recent scuba studies (Bridger 1970) demonstrated that otter boards and foot ropes of trawls can make recognizable tracks on the sea floor, and observations from a submersible in a heavily fished area of the northwest Atlantic (Caddy and

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Iles 1972) revealed a high frequency of tracks presumed to have been made by otter trawl boards. Some incidental effects of trawling on benthos and diet of demersal fish were observed by Arntz and Weber (1970).

Underwater studies by Medcof and Caddy (1971) showed that tracks made by clam dredges may persist for several days on sand and longer periods on mud, while preliminary observations on tracks of scallop dredges on sand (Caddy 1968) showed that this gear has a low efficiency and may cause appreciable incidental mortalities that attract predators into the tracks. This paper further describes and attempts to quantify some incidental effects of fishing over a scallop ground.

Materials and Methods

Area of Investigation

The study area was the north side of Chaleur Bay, off Paspébiac, Québec (48°00′N 65°15′W), at 40–50 m depth. A small fishery landing 80–90 metric tons of scallop meats per year began in Chaleur Bay in 1969 (Anon. 1971) and gave employment to a fleet of small inshore scallopers, equipped with gangs of 3 or 4 Alberton style drags (Bourne et al. 1965). A small inshore fishery for groundfish also operates in the area.

The substrate on the scallop grounds is sand overlaid by glacial gravel, 1–10 cm in diameter, coated with *Lithothamnion*. Occasional boulders up to 60 cm across are embedded in the gravel. Except for *Placopecten magellanicus*, large epibenthic species were present at a low density: the most common forms being *Metridium* sp., *Cucumaria frondosa*, *Strongylocentrotus drobachiensis*, and the starfish *Leptasterias polaris*, *Solaster endeca*, and *Crossaster* (Solaster) *papposus*.

Fishing Gear and Procedures

Two types of dredges were used in the study. The principal one was an 8-ft (2.4-m) wide offshore dredge (Bourne 1964), weighing 0.6 metric tons empty, with a bag knit from 76-mm (internal diameter) steel rings. The other was a gang of three 0.8-m wide Alberton inshore dredges, used locally and considered by fishermen to catch fewer rocks on rocky ground than the offshore "chain sweep" dredge. Several of these dredges are linked together by a common steel tow bar at the front and a wooden dumping bar behind, the upper and lower edges of each dredge mouth being armed with blunt teeth 4 cm long. Only qualitative observations were made on tracks of this type of gear.

Tows of 0.8–1.2 km were made parallel to the coast in a roughly E–W direction. A marker buoy was laid at start and finish of each tow. Underwater observations were made on the dredge tracks from the Perry submersible PC8 (Fig. 1A), a two-man vehicle. After diving about 400 m from the track, a N–S traverse was made across bottom in the PC8 to intersect it. Early attempts to locate the unmarked dredge track were frustrated by existing grooves on the sea floor left by past otter trawling activities in the area. Hence, it was necessary to mark the track by attaching to the dumping bar of the dredge, before fishing, 600–700 m of $\frac{3}{8}$-inch (0.95-cm) lead core rope, which lay in the track at the end of the tow. After each tow, the dredge was left on bottom until the track had been inspected. The time interval between finishing a tow and inspecting the track was less than 1 hr. During dives 1–3, the frequency of old trawl tracks and abundance of predators outside the dredged area was noted. Dives 4 and 5, and 9–12 inclusive, were on tracks of the offshore dredge, dives 6–8 inclusive were on tracks of the inshore dredge, and dives 9 and 10 were aborted due to odometer malfunction.

Data Recording and Equipment

Visual observations, made through the curved plexiglas window at the front of the submersible (Fig. 1A), were recorded on magnetic tape together with time checks at the start and finish of each dive, and observations on course, depth, and bottom terrain. An odometer wheel (Fig. 1B) measured the distance travelled on bottom. At each wheel revolution, a sonic switch superimposed a 2900-Hz tone onto the magnetic tape.

Photography

Still (35-mm) and movie (16-mm) records were made through the plexiglas viewport, and an EG&G camera and flash unit were attached with the lens 0.8 m off bottom, pointing downward at 45° to the vertical to take pictures of the sea floor directly in front of the viewport at 7-sec intervals. This camera was later calibrated in sea water while mounted at the same height and angle above a 2 × 2-m board ruled in 5-cm squares. The estimated area of sea floor visible in the photographs was 0.54 m^2. Counts of scallops and fish and predatory invertebrates were made from the bottom photographs; separate records were kept for photographs in which the rope marking the dredge track was visible.

Visual Observations

Observations were of three types: (a) scallop abundance and any obvious effects of fishing on the scallop stocks and the ground; (b) abundance of fish and predatory invertebrates both on and off the tracks; and (c) marks on the sea floor apparently caused by fishing prior to the experiment.

Only two of these phenomena were assessed at a time (Table 1) and, to avoid observer fatigue, the dives were divided into sections of 2.5–15-min duration, during each of which uninterrupted observations were made on one or two of the above parameters (Table 1). Distance travelled during each section was determined from its duration and the average ground speed of the submersible. Average speed was determined from the tape record by timing the frequency of rotation of the 135-cm circumference odometer wheel, and ranged from 0.17–0.55 m/sec. This indirect method of estimating the distance travelled has to be employed, rather than the

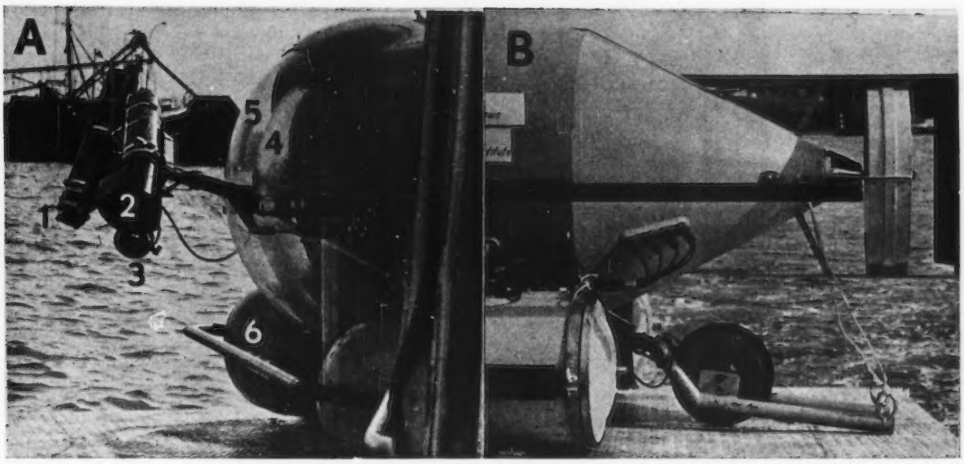

FIG. 1. Fore and aft views of PC8 submersible. (A) EG&G camera (1), flash unit with trough reflector (2), and flood light (3), are suspended from dive plane support, directly in front of acrylic window (4), with its outer free-flooding acrylic shield (5). Below window, a calibrated bumper bar connects two battery pods (6). (B) Odometer wheel, rotating in a free-flooding pipe framework, is slung below main propeller directly behind battery pod.

FIG. 2. Groove on sea floor considered to have been made by otter trawl door. (Long arrow indicates direction of tow; short arrows point to spoil ridge on one side of groove.)

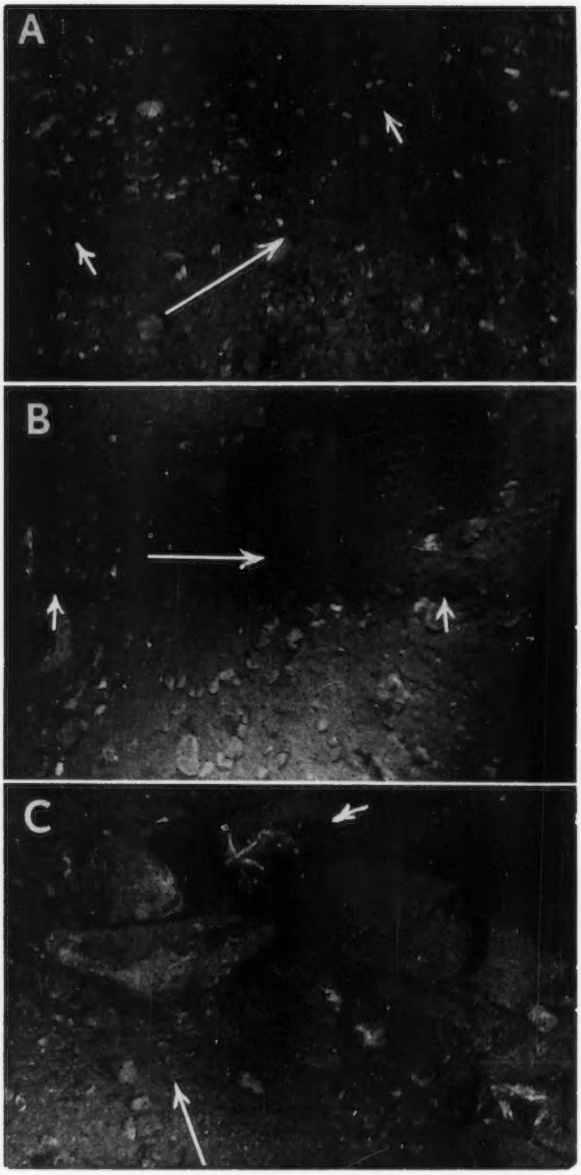

FIG. 3. Track of offshore dredge: (Long arrows indicate direction of tow) (A) Edge of track left by offshore dredge (short arrows). (B) Groove in centre of offshore dredge track left by tow bar (short arrows). (C) Boulders dislodged by offshore dredge; long arrow shows direction of tow; short arrow indicates overturned boulder with *Leptasterias polaris*; *Cancer irroratus* in right foreground.

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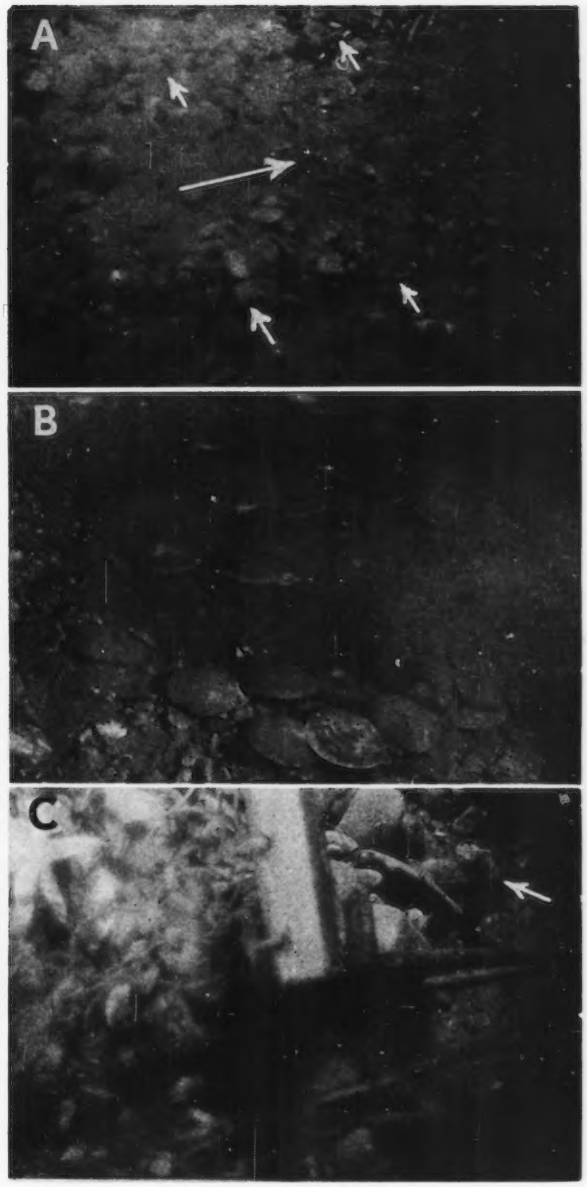

FIG. 4. Track of Alberton dredge (long arrow indicates direction of tow). (A) Spoil ridges between tracks made by individual dredges (short arrows). (B) Pile of 15–20 large (120–150 mm) scallops within area of 2–3 m^2 inside dredge tracks; it is presumed that these fell out of the dredge during the tow. (C) Alberton dredges stationary on bottom at end of tow; short arrow indicates spoil heap in front of dredge mouth.

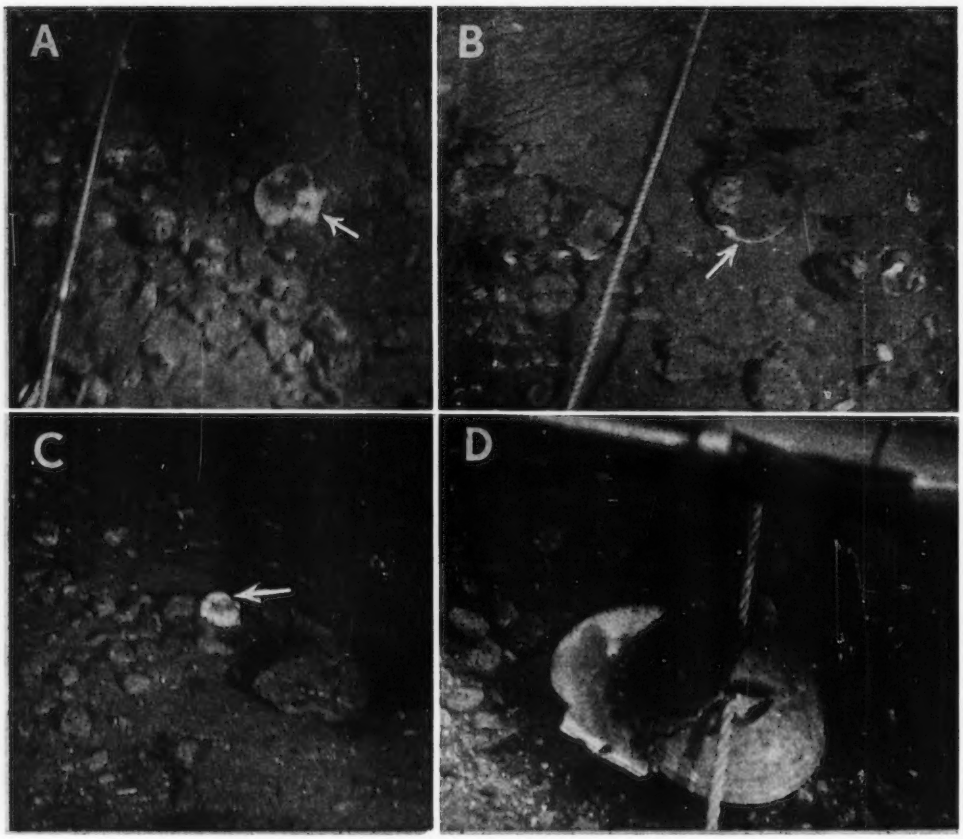

FIG. 5. Some effects of fishing on scallops left in dredge tracks (rope marks center of track). (A) Scallop on edge between two boulders. (B) "Non-lethal" breakage of shell margin. (C) Scallop partly buried in sediment. (D) Lethally damaged scallop (broken hinge), being eaten by *Cancer irroratus*.

more accurate method of counting the total number of revolutions per section (Caddy 1970), since the odometer tone was of variable strength and was occasionally masked by the verbal record. During dive 4 and parts of dive 5, the odometer tone was completely lacking due to odometer malfunction; in dive 4, the arithmetic mean of speed (0.37 m/sec) for all other dives was used and, in dive 5, the speed of the submersible during the first section of that dive was considered to apply to later dive sections.

Because of the apparent reduction in size caused by the convex viewport of the PC8, dimensions of objects on the sea floor were estimated as they passed under the bumper bar (Fig. 1A) 30 cm off bottom. A 1½-m scale graduated in 10-cm units was attached to its upper surface. It was estimated from this that a 0.75-m wide swath of bottom was scanned visually.

Results

Observations on the Effects of Gear on the Sea Floor

Trawl tracks — Characteristic grooves in the sea floor were seen during dives 1, 2, 10, and 12, extending in straight lines in a predominantly E–W

Table 1. Dive durations, distances travelled, and types of observation (*) made from the submersible inside and outside tracks made by scallop dredges.

Dive	Date	Dive duration (*min*)	Dive section	Duration of dive section (*min*)	Estimated distance travelled (*m*)	Type of observation: Trawl track frequency	Scallop abundance & damage	Predator abundance
				Observations outside the dredge tracks				
1	16/8/71	65	a	4.1	86	*		*
			b	5.4	101	*		*
			c	7.5	192	*		
2	16/8/71	88	a	2.8	77	*		*
			b	3.8	113	*		*
			c	3.9	95	*		*
			d	6.0	177	*		
			e	9.2	211	*		*
			f	9.6	253	*		*
			g	3.5	84	*		
3	16/8/71	46	a	3.3	98		*	*
4	17/8/71	60	a	2.8	59	*		*
			f	10.3	216		*	*
5	17/8/71	75	a	5.6	71		*	*
			b	2.5	94			*
11	19/8/71	41	a	7.8	153		*	*
12	19/8/71	96	a	4.5	67		*	*
Totals		7 hr 51 min		1 hr 33 min	2147 m	1448 m	605 m	1694 m
				Observations in the dredge tracks				
4	17/8/71	60	b	4.3	96		*	*
			c	5.9	132		*	*
			d	14.7	327		*	*
			e	2.9	65		*	*
5	17/8/71	75	c	5.0	165		*	*
			d	3.8	124		*	*
			e	4.0	130		*	*
			f	5.0	163		*	*
11	19/8/71	41	b	5.7	576		*	*
			c	12.3	147		*	*
12	19/8/71	96	b	10.3	148		*	*
			c	7.9	128		*	*
			d	8.1	141		*	*
Totals		4 hr 32 min		1 hr 29 min	2342 m		2342 m	2342 m

direction. These closely resembled marks described by Arntz and Weber (1970) that were considered to have been made by otter trawl doors (Fig. 2). The grooves cut several centimeters through the surficial gravel, exposing the underlying sand, and were typically deeper on one side of the groove than the other, with a spoil ridge next to the deep side of the depression. The widths of 41 tracks ranged from 15 to 70 cm (mean width, 36±11 cm), which is narrower than either the 2–4 gang scallop dredges used locally (1.6–3.2 m wide) or the doors (2.3 m long) for the Yankee 35 and 36 trawls most commonly used in the Gulf of St. Lawrence (Carrothers and Foulkes 1972). Assuming an attack angle of 35° for the doors (Carrothers personal communication), the doors should leave a track 1.3 m wide if in contact with the bottom along their whole length. This is wider than any of the tracks observed, but it seems likely that on hard sediments only the heavier trailing edge of the door scours the bottom, leaving a narrow track with a wedge-shaped cross section.

These tracks were abundant, adjacent tracks being separated by a mean distance of 11.4 m, reaching a maximum of one for every 5 m of travel during dive 1. Making the simplest assumption, namely, that the 36-cm wide tracks were crossed at right angles, provides us with minimum estimates of the area of sea floor disturbed, 3 and 7%, respectively. No estimate could be made of the age of the tracks, but it is unlikely that they were very recent, since no trawlers were seen in the area during the study.

Dredge tracks — Suspended sediment reduced underwater visibility from 4–8 m to less than 2 m within 20–30 m of the track, but dispersed within 10–15 min of the tow, coating the gravel in the vicinity of the track with a thin layer of fine silt and obscuring the pink-mauve colour of the *Lithothamnion*.

Offshore dredge — The track of the offshore dredge had a flat floor and increased in depth from scarcely below the sediment surface at the beginning of the tow to several centimeters deep at the end. Gravel fragments were less frequent inside the track (Fig. 3A) and many were overturned, showing their grey underside. Rocks 20–40 cm in diameter were dislodged every 10 m of track during dive 4 and every 30 m during dive 12. Some boulders had been overturned (Fig. 3C), others ploughed along leaving a groove several meters long. The empty holes left by some of the rocks were clearly evident. Over areas of sand and fine gravel, marks left by individual dredge rings showed clearly, as did a narrow depression left by the tow bar of the dredge in the center of the track (Fig. 3B). The edge of the track was sometimes marked by an impression left by the lateral skids, although there was rarely any sign of the "heaping" of sediments noted on the trawl tracks.

Inshore dredge — This type of gear is usually towed on a very short warp with the spreader bar off bottom so that the leading surface of the teeth must make an oblique angle with the bottom. An explanation for the selectivity of the gear may be that scallops ride up the inclined teeth into the dredge, while heavy rocks deflect the teeth back beyond the vertical so that the dredge rides over them.

The tracks left by the Alberton dredge, like those for the offshore dredge, were shallow with a flat floor. The gravel was sparser inside than outside the track and dislodged boulders were commonly observed. Tooth marks were seen over sandy bottom. The tracks could be distinguished from those of the offshore dredge by the spoil ridges left between adjacent dredges (Fig. 4A). Piles of small rocks were seen at intervals along the track and, on at least three occasions, 15–20 large scallops were observed apparently deposited within an area of 1–2 m^2 (Fig. 4B). Observations at the dredge (Fig. 4C) showed that small rocks had been "bulldozed" along in front of the dredge. Alberton dredges have a relatively small capacity and these observations suggest that, when they are full, contact of the teeth with any fixed obstacle tips the dredge forward and dumps its contents onto the bottom. This presumably may occur when enough rocks have accumulated in front of the teeth to impede progress of the dredge.

Effects of Dragging on Scallops Left in the Track

Any apparent effects of fishing on scallops in the drag path were noted; "lethal" and "nonlethal" shell breakages were recorded separately (categories A and B of Table 2) using criteria suggested by Medcof and Bourne (1964). The most frequent cause of mortality noted was hinge breakage (Fig. 5D). Breakage of the shell margins was the most common type of "nonlethal" injury (Fig. 5B). Undisturbed scallops (category E, Table 2) usually occupy slight depressions in the bottom, formed by mantle rejection currents, and individuals overturned or dislodged from these depressions by the dredge are included in category D of Table 2. Animals partly buried (Fig. 5C) or wedged between or under rocks (Fig. 5A) were placed in category C. A note was also made of swimming scallops (category F); few individuals larger than 60-mm shell diameter were observed to swim.

TABLE 2. Numbers and condition of scallops left in the offshore dredge track after fishing.

	Dive no.					
	4	5	11	12	Total	%
A "Lethal" damage	26	8	1	0	35	10.8
B "Nonlethal" damage	6	4	1	3	14	4.3
C Buried or wedged under rocks	14	5	4	0	23	7.1
D Moved by dredge	24	9	10	26	69	21.4
E No signs of disturbance	48	43	43	25	159	49.2
F Swimming	9	3	6	5	23	7.1
Totals	127	72	65	59	323	

Wide variations in incidental mortalities were observed during dives over four different offshore dredge tracks; lethal and nonlethal breakages were more frequent on rocky ground (dive 4) than on sand (dive 12). However, the incidental mortality of 10–11% calculated for the four dives is probably an underestimate since only obvious signs of damage could be recognized; and it is likely that at least some scallops in categories B, C, and D were lethally damaged, and perhaps even some individuals in category E. Sublethal damage may still expose an injured animal to predation. A conservative estimate of the incidental mortality to uncaught scallops in the track is therefore thought to be in the range 15–20%. The estimated fishing efficiency of an 8-ft offshore dredge is approximately 15% (Caddy 1971) so that, from present data, at least 13–17% of all scallops in the path of the dredge must be lethally damaged but not caught. More long-term studies will be needed to determine if "sublethally" damaged scallops recover from injuries caused by dredging.

AGGREGATION OF FISH AND INVERTEBRATES IN THE DREDGE TRACKS

Previous studies (Caddy 1968; Medcof and Caddy 1971) described aggregations of predatory and scavenging fish and invertebrates in tracks of scallop and clam dredges shortly after fishing, but no quantitative estimates of predator abundance were made, although feeding behavior of several species was noted. In the present study, the relative frequencies of occurrence of six common species of fish and invertebrates was determined inside and outside the dredge tracks. Frequency was expressed per 100 m of travel separately for each dive section, counts being made during 2342 and 1694 m of travel inside and outside the track, respectively. (Frequencies of occurrence of scallops were only estimated in 605 m of travel outside the track, Table 1.) Population densities were also estimated from bottom photographs. The ratio of frequencies inside and outside the track is considered to represent the apparent "preference" of each species for the dredge tracks (Table 3). Although no method of defining confidence intervals for these ratios could be found, the three most active species were significantly more abundant within the tracks than outside. (The Mann–Whitney U test was used rather than the Students' t test to avoid the assumption of equal variances, which would have been unjustified in this case.) Winter flounders (*Pseudopleuronectes americanus*) had the highest frequency ratio of 30.3:1, but sculpins (*Myoxocephalus* sp.) and rock crabs (*Cancer irroratus*) were also more abundant inside the tracks than outside (frequency ratios of 5.5:1 and 3.6:1, Table 3). No significant differences were found between abundance of semisedentary forms such as starfish and scallops inside and outside the tracks, although all were less abundant in the dredge tracks than outside, probably because these animals, unlike more active species, are more susceptible to capture by the dredge and had not moved into the track in the 0.5–1-hr period since dragging was carried out.

Bottom topography was often hard to interpret from the photograph because of the small area (0.54 m^2) covered. This made it difficult to decide whether a photograph was taken inside or outside the tracks. Photographs containing segments of the marker rope could be identified with confidence as having been taken within the tracks but, because of the width of the track (2.4 m), some photographs in which the marker line was not seen must also have been taken inside the tracks. As a result, the ratio of frequencies, calculated in Table 4 from bottom photographs on and off the line, underesti-

TABLE 3. Calculated numbers of six common fish and invertebrates seen per 100 m of travel across bottom in dive sections inside and outside the dredge tracks. Dive sections ranked by frequency of occurrence (f), and differences between the mean frequencies ($\bar{f}$) inside and outside the track tested by Mann–Whitney U test.

	Calculated No./100 m out of track	Significant difference at $P=$	Calculated no./100 m in track	$\frac{\bar{f}\text{ in}}{\bar{f}\text{ out}}$
Scallop	14.3, 19.4, 30.1, 34.7, 102.8		3.4, 5.4, 6.2, 8.9, 9.9, 14.1,	
(*P. magellanicus*)	N[a] = 221, $\bar{f}$ = 40.3/100 m	N.S.	15.1, 15.5, 18.0, 20.6, 26.3, 35.2, 44.2	
			N = 356, $\bar{f}$ = 17.1/100 m	0.4
Sunstars	0.0, 0.0, 0.0, 0.0, 0.7, 0.8,		0.0, 0.2, 0.6, 0.7, 0.8, 0.8,	
(*S. endeca* and	0.9, 2.0, 2.1, 2.3, 2.8, 4.3,		0.8, 0.9, 1.2, 1.4, 1.5, 1.6,	
C. papposus)	5.3, 7.5		3.4	
	N = 31, $\bar{f}$ = 2.1/100 m	N.S.	N = 21, $\bar{f}$ = 1.1/100 m	0.5
Northern starfish	0.4, 0.4, 0.5, 0.8, 1.0, 1.1,		0.0, 0.0, 0.0, 0.0, 0.0, 0.0,	
(*L. polaris*)	1.3, 1.3, 1.4, 1.5, 1.7, 2.0,		0.6, 1.4, 1.4, 2.1, 2.3, 2.7,	
	2.3, 2.7		3.1	
	N = 27, $\bar{f}$ = 1.3/100 m	N.S.	N = 21, $\bar{f}$ = 1.1/100 m	0.8
Rock crab	0.0, 0.0, 0.0, 0.0, 0.9, 1.1,		0.5, 1.4, 1.6, 1.8, 2.1, 3.7,	
(*C. irroratus*)	1.1, 1.2, 1.3, 1.4, 2.0, 3.0,		4.0, 4.0, 7.1, 7.7, 9.4, 17.4,	
	3.1, 8.5		17.6	
	N = 23, $\bar{f}$ = 1.7/100 m	0.02	N = 137, $\bar{f}$ = 6.0/100 m	3.6
Sculpin	0.0, 0.0, 0.0, 0.0, 0.0, 0.0,		0.0, 0.0, 0.0, 1.0, 1.1, 2.2,	
(*Myoxocephalus* sp.)	0.5, 0.8, 0.9, 1.1, 1.2, 1.3,		3.1, 3.1, 4.2, 7.9, 8.5, 12.2,	
	2.6, 2.8		13.5	
	N = 15, $\bar{f}$ = 0.8/100 m	0.05	N = 103, $\bar{f}$ = 4.4/100 m	5.5
Winter flounder	0.0, 0.0, 0.0, 0.0, 0.0, 0.0,		0.0, 0.3, 3.1, 6.8, 7.3, 8.6,	
(*P. americanus*)	0.0, 0.0, 0.5, 0.9, 1.1, 2.0,		12.1, 16.0, 16.9, 20.9, 43.7,	
	2.0, 2.3		49.0, 63.1	
	N = 11, $\bar{f}$ = 0.6/100 m	0.002	N = 337, $\bar{f}$ = 19.1/100 m	30.3

[a]N = total no. observed.

TABLE 4. Estimated population density of six common species of fish and invertebrates from bottom photographs (combined observations: all dives).

Species	Marker rope showing (254 frames)		No marker rope showing (738 frames)		Frequency ratio (on/off rope)
	No. observed	No./100 m^2	No. observed	No./100 m^2	
Scallop	32	23.3	137	34.4	0.7
Sunstars	3	2.2	8	2.0	1.1
Northern starfish	10	7.3	17	4.3	1.7
Rock crab	17	12.4	17	4.3	2.9
Sculpin	10	7.3	5	1.3	5.6
Winter flounder	21	15.3	23	5.8	2.6

mates the difference between population densities inside and outside the track. Thus, the range of frequency ratios determined photographically was only from 0.7–5.6, while that determined visually was from 0.4–30.3. Despite this, with the exception of winter flounder, which ranked third instead of first in apparent preference of the tracks, ranking remained unchanged. The visual observations are more valuable than the photographs in determining relative frequencies on and off the track because: (a) they provided the only reliable method of determining the track boundaries; (b) they gave greater coverage both in area and in number of individuals observed; and (c) flounders and sculpins were often observed to swim out of the path of the submersible before entering the camera field directly in front of the bumper bar. The photographs were, however, valuable in estimating the population densities of the less active forms, and these were comparable with those determined in a similar study in the southern Gulf of St. Lawrence (Caddy 1970); densities of most large epibenthic species in the Bay of Chaleur were in the range of 1–6 individuals/100 m^2 (Table 4).

A deficiency of these observations on predator aggregation is the lack of population density estimates before fishing. As a result, the extent to which initial "background" densities were depleted by movements of predators into the tracks is not known. However, on the first three dives outside the immediate vicinity of the dredged area, frequencies of observation of flounders, sculpins, and rock crabs were 0.5, 1.2, and 0.9 individuals/100 m travelled, comparable with the average densities observed outside the tracks on later dives. It seems reasonable, therefore, to assume that the background densities of predators prior to fishing were not much higher than those observed outside the tracks following fishing so that the relative frequencies inside and outside the tracks do measure the preference of active predators for fished over unfished areas.

Discussion

Effects of Fishing on the Bottom

The bottom type and hydrographic regime in Chaleur Bay probably allow marks made by fishing gear to remain recognizable for a long time. Tidal currents faster than 1 km/hr were not encountered in the area and this speed is insufficient to redistribute gravel larger than 1 cm in diameter. Even a relatively minor fishery may have a significant cumulative effect on bottom microtopography under these conditions.

In this area, it was possible to distinguish between tracks presumed left by otter trawls and by both types of scallop dredges tested. Both scallop dredges contribute to an appreciable roughening of the bottom, lifting large boulders from the sediment and overturning many of them, presumably leading to destruction of the epifuana on their upper surfaces. Under conditions of strong tidal flow, one suspects that intensive dredging will lead to the erosion of sediments lifted into suspension by the dredge, but systematic studies under different sedimentary and hydrographic conditions are called for to elucidate the possible magnitude of such effects.

Dredge Performance and Design

Considerable improvements are possible in the design of existing scallop dredges, both (a) to improve efficiency and selectivity, and (b) to reduce incidental mortalities due to fishing. These observations support earlier studies on selectivity (Caddy 1971), which suggested that most selection occurs through the belly of the offshore dredge. The high incidental mortality in the tracks was probably caused by the dredge passing over scallops that had either gone underneath the sweep chain or passed through the meshes in the dredge belly. An improvement in selection and reduction in incidental mortalities might result from raising the belly of the dredge slightly off bottom.

The need for underwater observations in developing fishing gear is illustrated by the Alberton dredge, which is highly inefficient, filling and dumping itself several times during a single 0.8–1 km tow.

Aggregation of Predators

The pronounced and rapid aggregation of foraging fish in the dredge tracks poses questions as to the sensory and behavioral factors underlying this phenomenon, as well as its role in the trophic ecology of bottom-feeding fish and possible use in increasing the efficiency of commercial fishing operations.

The formation of feeding aggregations by groundfish in response to a local abundance of food is a natural phenomenon and occurs in the absence of fishing operations. Population densities of red hake (*Urophycis chuss*), spiny dogfish (*Squalus acanthias*), sculpin (*Myoxocephalus* sp.), and skate (*Raja* sp.) were noted on beds of herring spawn by Caddy and Iles (1972); these were well in excess of those observed in their immediate vicinity. Feeding migrations of winter flounders into the intertidal zone were observed by Tyler (1971). Indirect evidence of the importance of bottom fishing in the trophic ecology of groundfish was presented by

Arntz and Weber (1970), who noted that the large, heavy-shelled bivalve *Cyprina islandica* formed a substantial component of the food of cod and flatfish in Kiel Bay only after trawling began in the area, and concluded that the fish were feeding on molluscs crushed by otter trawl board. This hypothesis appears to be confirmed by observations of Medcof and Caddy (1971), who observed cod and flounders feeding on broken *C. islandica* in the tracks of an hydraulic clam dredge, and by Margetts and Bridger (1971), who observed dabs foraging in trawl tracks. Further studies on the sensory and behavioral aspects of groundfish aggregation seem called for.

An impression of the potential importance of commercial dredging on the diet of bottom fish can be obtained from fishery statistics for Georges Bank, the largest scallop ground in the northwest Atlantic. A fleet of 80 large offshore vessels, each towing two 3.8-m wide dredges, towed an estimated 1.01×10^6 km across bottom in 1970. The cumulative area swept in the process was approximately 7700 km^2. Even with some repetitive fishing over the same area, a considerable fraction of the 37,000 km^2 of the scallop grounds must be swept over in a year, thus making a potentially large contribution to the energy budgets of bottom foraging fish.

The author was unable to locate any published references on aggregation of fish in an area following fishing. Possibly, the range of detection of gear tracks by fish is short enough that it merely leads to redistribution of fish populations within the fished area and not to a local increase in stock. However, the empirical observation that the first trawl set in an area is poorer than subsequent ones appears to be widely reported amongst fishermen; consequently this phenomenon may merit further investigation by comparative fishing trials.

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ANON. 1971. La pêche aux pétoncles au Québec (Saison 1970). Bureau de la Statistique du Québec, Service des Statistiques de la Production, Division des Industries Primaires (Pêcheries), Juillet 1971. 26 p.

ARNTZ, W. E., AND W. WEBER. 1970. *Cyprina islandica* L. (mollusca, Bivalvia) als Nahrung von Dorsch und Kliesche in der Kieler Bucht. Ber. Dt. Wiss. Komm. Meeresforsch. 21(1–4): 193–209.

BOURNE, N. 1964. Scallops and the offshore fishery of the Maritimes. Fish. Res. Board Can. Bull. 145: 60 p.

BOURNE, N., E. I. LORD, AND A. R. MCIVER. 1965. Gulf of St. Lawrence scallop survey — 1961. Fish. Res. Board Can. MS Rep. 807: 52 p.

BRIDGER, J. P. 1970. Some effects of the passage of a trawl over the sea bed. ICES Gear Behav. Comm. Meet. Doc. B10: 18 p.

CADDY, J. F. 1968. Underwater observations on scallop (*Placopecten magellanicus*) behaviour and drag efficiency. J. Fish. Res. Board Can. 25: 2123–2141.

1970. A method of surveying scallop populations from a submersible. J. Fish. Res. Board Can. 27: 535–549.

1971. Efficiency and selectivity of the Canadian offshore scallop dredge. ICES Shellfish Benthos Comm. Meet. Doc. K25: 8 p.

CADDY, J. F., AND T. D. ILES. 1972. Underwater observations on herring spawning grounds on Georges Bank. Int. Comm. Northwest Atl. Fish. Res. Bull. (In press)

CARROTHERS, P. J. G., AND T. J. FOULKES. 1972. Trawl measurements. How Canadian east coast otter trawls behave. Fish. Res. Board Can. Gen. Ser. Circ. 57: 10 p.

GRAHAM, M. 1955. Effect of trawling on animals of the sea bed. Pap. Mar. Biol. Oceanogr. Deep Sea Res. Suppl. 3: 1–6.

MARGETTS, A. R., AND J. P. BRIDGER. 1971. The effect of a beam trawl on the sea bed. ICES Gear Behav. Comm. Meet. Doc. B8: 9 p.

MEDCOF, J. C., AND N. BOURNE. 1964. Causes of mortality of the sea scallop, *Placopecten magellanicus*. Proc. Nat. Shellfish Ass. 53: 33–50.

MEDCOF, J. C., AND J. F. CADDY. 1971. Underwater observations on performance of clam dredges of three types. ICES Gear Behav. Comm. Meet. Doc. B10: 7 p.

TYLER, A. V. 1971. Surges of winter flounder, *Pseudopleuronectes americanus*, into the intertidal zone. J. Fish. Res. Board Can. 28: 1727–1732.

Fatty Acid Composition of *Nephtys incisa* and *Yoldia limatula*

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Samples of the infaunal invertebrates *Nephtys incisa* and *Yoldia limatula* from Narragansett Bay, Rhode Island, have been analyzed for their fatty acid distribution. Based on total fatty acids, *Yoldia* contains 9–16% of an acid tentatively identified as 22:2. The ratios of 18:1/18:0 and 18:1/20:1 fatty acids of *Nephtys* from a polluted station in the bay are lower than the corresponding ratios for animals from relatively clean areas.

FARRINGTON, J. W., J. G. QUINN, AND W. R. DAVIS. 1973. Fatty acid composition of *Nephtys incisa* and *Yoldia limatula*. J. Fish. Res. Board Can. 30: 181–185.

Nous avons déterminé la répartition des acides gras sur des échantillons d'invertébrés de l'endofaune de la baie de Narragansett, Rhode Island, *Nephtys incisa* et *Yoldia limatula*. Sur la base des acides gras totaux, Yoldia contient de 9 à 16% d'un acide identifié provisoirement comme 22:2. Les proportions de 18:1/18:0 et 18:1/20:1 des acides gras chez *Nephtys* provenant d'un endroit pollué de la baie sont moindres que les valeurs correspondantes chez des individus qui habitent des territoires relativement propres.

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THE fatty acid composition of marine organisms can be influenced by environmental factors as well as by the fatty acid composition of the organisms' diet (Malins and Wekell 1969). This has caused Lovern (1964) to state that attempts to give average fatty acid compositions for depot fats are unrealistic. Jeffries (1967) postulated that stress from pollution caused changes in the fatty acid composition of *Mercenaria mercenaria*. The recent work of Pocock et al. (1971) has shown that stage of maturity and starvation significantly alters the lipid composition in *Nereis virens*, an intertidal polychaete.

We have determined the fatty acid composition of *Nephtys incisa*, an infaunal worm, collected at several stations within Narragansett Bay, Rhode Island, to investigate the variability of fatty acid composition in this estuarine species. Based on sediment hydrocarbon values (Farrington and Quinn 1973), the stations sampled range from polluted to relatively clean. We have also determined the fatty acid composition of a few samples of the bivalve, *Yoldia limatula*, which were collected with the polychaete samples.

Materials and Methods

The stations sampled are shown in Fig. 1. Samples of *N. incisa* and *Y. limatula* were obtained by wet-sieving sediment collected with a Foster anchor dredge. The organisms retained on a 2-mm screen were kept in bay water until arrival at the laboratory where they were either analyzed immediately, freeze-dried and then analyzed, or, in one case, frozen at –20 C overnight and analyzed the following day. *N. incisa* samples were obtained at three stations (E_2, D, C) each month for several months to test seasonal variation in fatty acid composition.

Several whole *Nephtys* or *Yoldia* were placed in 25-ml centrifuge tubes with teflon-lined screw caps. Ten milliliters of 0.10-N KOH in methanol, and 5.0 ml of benzene were added to each tube; the tubes were flushed with N_2, sealed, and heated at 100 C for 30 min with shaking every 10 min. The saponified samples were cooled, 5.0 ml of 0.3-N HCl was added, and the tubes were shaken and centrifuged. The benzene layers were drawn off and transferred to pear-shaped flasks, and the aqueous methanol phases were twice re-extracted with 5.0-ml

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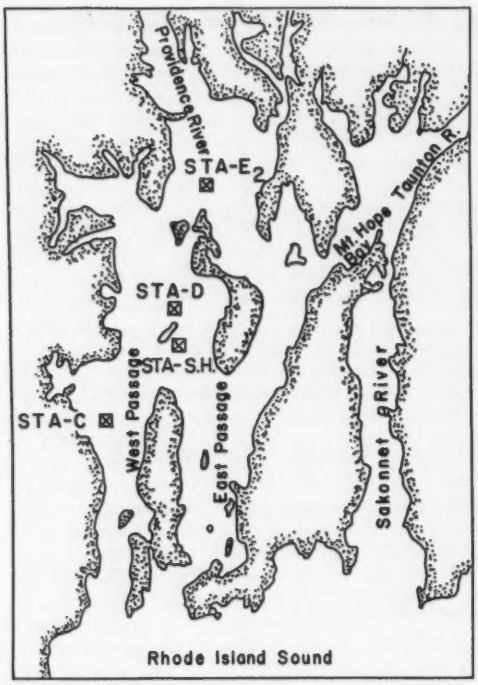

FIG. 1. Sampling stations, Narragansett Bay, Rhode Island.

portions of petroleum ether. The petroleum ether–benzene extracts were evaporated to dryness under reduced pressure.

The extract residues were transferred to screw-cap centrifuge tubes with teflon-lined caps, using 1 ml of methanol and 1 ml of benzene. Two milliliters of BF_3-methanol was added to each tube; the tubes were flushed with N_2, sealed, and heated at 100 C for 5 min. Methyl esters were isolated by adding 5 ml of water to each tube and extracting with three 5-ml portions of petroleum ether. Solvent was evaporated under reduced pressure, and the esters were purified by preparative thin-layer chromatography using predeveloped (chloroform/methanol, 1/1), 0.5-mm thick, 20 × 20-cm silica gel G plates developed in 90:10:1 petroleum ether:ethyl ether:acetic acid. Fatty acid methyl esters were analyzed on a 2.1-m 15% stabilized DEGS on Gas Chrom P-80/100 mesh column, a 1.8-m 17% stabilized DEGS on Chromosorb W-100/120 mesh column, a 1.8-m 10% UCCW-982 (Hewlett Packard silicone gum rubber) on Chromosorb W-80/100 mesh column, and a 0.7-m 2% OV-1 on Anakrom Q-100/110 mesh column. All columns were 3.2-mm outside diameter (2.2-mm inside diameter) stainless steel and operated with nitrogen carrier gas at 40 P.S.I.G. and a flow rate of 50–75 ml/min. The DEGS columns were used for routine analyses of fatty acid methyl esters, and these as well as the nonpolar columns were operated isothermally at 180 or 200 C. Hewlett Packard Model 700 and F and M Model 1609 Gas Chromatographs, both equipped with flame ionization detectors, were used in these analyses.

Fatty acid methyl esters of representative samples were qualitatively identified by comparison of their relative retention times with those of standard methyl esters on both polar and nonpolar columns before and after hydrogenation. The concentrations of fatty acids in selected samples were determined by incorporating a fatty acid internal standard (17:0[3]) into the extraction tube with the sample prior to saponification. The peak area (height times width at half height) of the internal standard methyl ester was then compared with the peak areas of the sample methyl esters on the gas chromatograms. Comparison of relative quantities, before and after hydrogenation, gave good agreement. All solvents used were distilled and blanks of solvents and reagents showed negligible quantities of fatty acids.

Quantitative results with National Heart Institute Fatty Acid Standard mixture D agreed with the stated composition data with a relative error of less than 5% for major components (>10% of total mixture) and less than 10% for minor components (<10% of total mixture)

[3]17:0 occurs in only trace quantities in *N. incisa* and *Y. limatula*. All fatty acids are given by normal carbon chain length:number of double bonds.

TABLE 1. Fatty acid weight percentage composition for *Nephtys incisa* and *Yoldia limatula* sampled at various stations and dates.

Fatty acid[a]	*N. incisa* Station D 10/28/70	*Y. limatula* Station E_2 11/25/70	*Y. limatula* Station E_2 3/10/71	*Y. limatula* Station S.H. & D 3/10/71
			(7)[b]	(6)[b]
14:0	trace	2.0	4.0	5.8
16:0	16.2	9.4	12.1	15.7
16:1	trace	2.7	9.4	18.2
18:0	13.1	11.4	14.2	13.4
18:1	16.1	11.3	15.9	15.7
20:1	8.8	13.0	9.9	7.6
20:4	1.2	2.8	4.3	2.2
20:5	23.2	11.4	11.1	8.9
22:1	n.d.[c]	3.6	–	–
22:2	n.d.	15.8	11.5	8.9
22:4	2.6	1.9	–	–
22:5	4.7	2.5	–	–
22:6	14.2	12.4	7.5	3.7
Concentration (*mg/g dry weight*)			8.84	10.12

[a]Normal carbon chain length:number of double bonds.
[b]No. of *Yoldia* per sample, samples of S.H. and D combined.
[c]Not detected.

(Horning et al. 1964). In addition, values obtained on analysis of a secondary reference standard (cod liver oil) were in the range cited by Ackman and Burgher (1964).

Results

The typical fatty acid distribution for marine animals (Gunstone 1967) was found, with the exception of the fatty acid tentatively identified as 22:2 (Table 1). A substantial amount of this fatty acid was present in the *Yoldia* samples. The equivalent chain lengths for this acid were 23.9 on DEGS, 21.3 on OV-1 before hydrogenation, and 22.0 on both columns after hydrogenation. This fatty acid is not 22:1 nor 22:4, based upon co-injection with authentic samples. Its elution from the DEGS column just after 22:1 suggests that it is 22:2.

There was no predictable seasonal variation in the composition of the major fatty acids of *N. incisa* from the three stations (E_2, D, C) sampled over a 7-month period (Table 2). The small size of the

TABLE 2. Fatty acid composition of *Nephtys incisa* sampled at various stations and dates.

Date		Weight percentage composition[a] 16:0	18:0	18:1	20:1	20:5	22:6	Concentration (*mg/g dry wt.*)
				Station E_2 (Depth = 6.1 m)				
8/19/70[b]		21.9	13.4	17.5	15.0	22.6	9.7	
		21.3	12.6	14.7	14.4	25.0	11.7	
9/25/70		21.9	13.3	15.0	12.9	28.3	8.4	
10/28/70		16.5	16.4	14.7	10.6	24.9	17.0	19.33
11/25/70		14.0	13.8	13.2	9.2	26.6	22.8	
2/10/71		35.6	11.6	14.6	10.7	15.0	12.5	11.59
3/10/71		16.7	16.2	16.5	10.4	24.0	16.2	
	Avg	21.1	13.9	15.2	11.9	23.8	14.0	
	SD	6.6	1.7	1.3	2.1	4.0	4.6	
				Station D (Depth = 7.0 m)				
8/19/70[b]		19.5	12.4	19.5	11.0	25.1	12.2	
		19.4	11.3	19.4	12.1	23.8	13.6	
10/28/70		16.3	12.2	16.2	8.4	27.8	19.1	15.46
11/25/70		16.6	10.8	15.0	9.7	28.3	19.2	
1/05/71		13.2	8.4	12.7	9.3	31.8	23.5	
2/10/71		22.3	12.1	24.4	9.7	18.6	12.9	9.00
3/10/71		21.7	15.9	20.1	9.4	20.2	12.7	
	Avg	18.4	11.9	18.2	9.9	25.1	16.2	
	SD	3.0	2.1	3.6	1.1	4.3	4.1	
				Station S.H. (Depth = 7.6 m)				
1/27/71		20.1	14.9	19.2	11.6	20.9	13.2	
2/10/71		23.2	13.1	21.9	9.3	20.3	12.2	9.95
3/10/71		21.7	15.9	20.1	9.3	20.1	12.7	
	Avg	21.7	14.6	20.4	10.1	20.4	12.7	
	SD	1.3	1.2	1.1	1.1	0.3	0.3	
				Station C (Depth = 3.0 m)				
8/19/70[b]		17.3	10.3	15.0	9.5	29.1	18.4	
		21.1	11.8	18.4	10.1	24.2	14.2	
9/25/70		19.4	8.4	15.8	7.5	33.9	15.0	16.52
10/28/70		20.4	11.8	15.5	9.1	25.9	17.3	
11/25/70		16.7	10.7	15.7	8.9	28.5	19.3	
2/10/71		26.3	14.3	22.7	10.4	15.8	10.8	9.76
	Avg	20.2	11.2	17.2	9.3	25.8	15.8	
	SD	3.1	1.8	2.7	0.9	5.4	2.9	

[a]Only the major fatty acids listed were used to calculate weight percent composition for this table.
[b]Samples split and analyzed in duplicate.

sample and short duration of the program may have prevented the detection of small variations. However, there were no large-scale variations among the major fatty acids.

Major fatty acids of *Yoldia* from two depositional environments appear to show a decrease in abundance of long-chain polyunsaturated acids at Stations S.H. and D (samples combined) relative to Station E_2, but an increase in 16:0 and 16:1.

Examination of fatty acid composition of *Nephtys* from different stations (Table 2) showed that there might be a difference in the 18:1/18:0 and 18:1/20:1 ratios. These ratios were calculated for all the sampling dates and are presented in Table 3. The ratios were compared using the "*t*" test on data paired by sampling dates (1% level) and combined data for all dates (5% level). There was a significant variation for both ratios between Stations E_2 and D, but no significant difference between Stations D and C and Stations E_2 and S.H. Station S.H. data are regarded as not representative of that station due to the small number of samples.

Some of the variation between stations and within stations for different sampling dates could have been due to differences in the fatty acid composition between different size-classes at the same station.

TABLE 3. Ratios of 18:1/18:0 and 18:1/20:1 fatty acids of *Nephtys incisa* from different depositional environments.

	Station							
	E_2		D		S.H.		C	
Date	18:1/18:0	18:1/20:1	18:1/18:0	18:1/20:1	18:1/18:0	18:1/20:1	18:1/18:0	18:1/20:1
8/19/70	1.21	1.09	1.65	1.69	n.s.[a]	n.s.	1.51	1.70
9/25/70	1.13	1.16	n.s.	n.s.	n.s.	n.s.	1.88	2.11
10/28/70	0.90	1.39	1.33	1.93	n.s.	n.s.	1.31	1.70
11/25/70	0.96	1.44	1.39	1.55	n.s.	n.s.	1.47	1.76
1/05/71	n.s.	n.s.	1.51	1.37	n.s.	n.s.	n.s.	n.s.
1/27/71	n.s.	n.s.	n.s.	n.s.	1.29	2.35	n.s.	n.s.
2/10/71	1.26	1.36	2.02	2.52	1.67	2.16	1.59	2.18
3/10/71	1.02	1.58	1.26	2.14	1.26	1.66	n.s.	n.s.
Avg	1.08	1.34	1.53	1.87	1.41	2.06	1.55	1.89
SD	0.13	0.17	0.25	0.38	0.20	0.29	0.19	0.21

[a]n.s., not sampled.

TABLE 4. Comparison of fatty acid weight percentage composition between size classes of *Nephtys incisa*.

							Ratio		
Station (*Date*)	16:0	18:0	18:1	20:1	20:5	22:6	18:1/18:0	18:1/20:1	Concentration[a]
S.H. (*1/27/71*)									
Small[b]	18.2	14.7	20.0	11.9	22.6	12.8	1.35	1.68	2.86
Medium	19.1	14.3	18.8	10.7	22.2	15.1	1.31	1.76	2.43
Large	23.1	15.8	19.0	12.3	18.3	11.7	1.20	1.54	1.80
Avg	20.1	14.9	19.3	11.6	21.0	13.2	1.29	1.66	2.36
SD	2.6	0.8	0.6	0.8	2.3	1.7	0.07	0.09	0.53
D (*1/5/71*)									
Small	13.5	8.8	8.9	8.9	33.4	23.1	1.39	1.00	4.73
Medium	13.2	8.5	14.6	9.0	31.8	23.0	1.72	1.62	4.67
Large	12.9	7.9	14.7	10.0	30.3	24.3	1.88	1.47	4.69
Avg	13.2	8.4	12.7	9.3	31.8	23.5	1.66	1.36	4.69
SD	0.3	0.5	3.3	0.6	1.6	0.7	0.29	0.26	0.07

[a]Concentration of fatty acids in mg/g dry weight of *Nephtys*. Difference between stations is attributed to more efficient blotting of Station D samples.

[b]Small = 2.0 cm or less; Medium = 2.0–3.0 cm; Large = greater than 3.0 cm in length.

No attempt was made to differentiate between classes for all the sampling dates because of the small number of samples available. Table 4 presents the available data and shows the difference in fatty acid composition attributable to differences in classes. The differences between size-classes probably accounted for a small part of the variation of *Nephtys* fatty acids from different stations and within stations on different dates.

Discussion

The small sample size for *Yoldia* precludes meaningful interpretation of the differences in fatty acid composition between stations. However, it is interesting to note that 22:2 is present in high concentrations (9 to 16% of the total fatty acid), which is atypical for marine organisms. It is found in trace quantities only or not reported for many marine organisms (Gunstone 1967), but accounts for approximately 8% of the fatty acids in *M. mercenaria* (Farrington 1971). The relatively high percentage of this fatty acid in these molluscs may be related to some unique biochemical pathway. Analyses of other bivalves are required to provide additional information.

The fatty acid composition of *Nephtys* exhibited a significant difference in the ratios of 18:1/18:0 and 18:1/20:1 between Station E_2 and Stations D and C. Based on fecal coliform counts, Station E_2 is in an intermittently polluted area (Rhode Island Department of Health, Division of Water Pollution Control, Providence, Rhode Island), and the effect of this pollution stress might cause the 18:1/18:0 and 18:1/20:1 ratios to be lower than those from the nonpolluted stations. The influence of the gut content of this polychaete on its fatty acid composition is thought to be negligible because its food, the marine sediment, has a low concentration of fatty acids relative to the *Nephtys* itself (Farrington and Quinn 1971).

The value of understanding the influence of homeostatic adjustment to stress on the composition of biochemical compounds has been discussed by Jeffries (1967). Such changes could be detected before the stress becomes too large and the organisms, or the community of which they are a part, are destroyed. Investigation of the fatty acid content of a particular lipid class, such as glycerides or phospholipids from organs of various benthic animals, may prove to be a more sensitive indicator of stress than the total fatty acid composition of the whole organism.

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ACKMAN, R. G., AND R. D. BURGHER. 1964. Cod-liver oil: component fatty acids as determined by gas–liquid chromatography. J. Fish. Res. Board Can. 21: 319–326.

FARRINGTON, J. W. 1971. Benthic lipids of Narragansett Bay — fatty acids and hydrocarbons. Ph.D. Thesis. Univ. Rhode Island, Kingston, R. I. 141 p.

FARRINGTON, J. W., AND J. G. QUINN. 1971. Fatty acid diagenesis in recent sediment from Narragansett Bay, Rhode Island. Natur.-Phys. Sci. 230: 67–69.

1973. Petroleum hydrocarbons in Narragansett Bay. Pt. I. Survey of hydrocarbons in sediment and clams (*Mercenaria mercenaria*). Estuarine and coastal marine science. (In press)

GUNSTONE, F. D. 1967. An introduction to the chemistry and biochemistry of fatty acids and their glycerides. Chapman and Hall, Ltd., Great Britain. 209 p.

HORNING, E. C., E. H. AHRENS, JR., S. R. LIPSKY, F. H. MATTSON, J. H. MEAD, D. A. TURNER, AND W. H. GOLDWATER. 1964. Quantitative analysis of fatty acids by gas-liquid chromatography. J. Lipid Res. 5: 20–27.

JEFFRIES, H. P. 1967. Chemical responses by marine organisms to stress. Grad. Sch. Oceanogr., Univ. Rhode Island, Kingston, R. I. Tech. Rep. 67–4: 76 p.

LOVERN, J. A. 1964. The lipids of marine organisms. Annu. Rev. Oceanogr. Mar. Biol. 2: 169–191.

MALINS, D. C., AND J. C. WEKELL. 1969. The lipid biochemistry of marine organisms. Progr. Chem. Fats Other Lipids 10: 475–486.

POCOCK, D. M.-E., J. R. MARSDEN, AND J. G. HAMILTON. 1971. Lipids in an intertidal polychaete and their relation to maturation of the worm. Comp. Biochem. Physiol. 39A: 683–697.

Genetics of Glycerol-3-phosphate Dehydrogenase Isozymes in White Muscle of Lake Whitefish (*Coregonus clupeaformis*)

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CLAYTON, J. W., W. G. FRANZIN, AND D. N. TRETIAK. 1973. Genetics of glycerol-3-phosphate dehydrogenase isozymes in white muscle of lake whitefish (*Coregonus clupeaformis*). J. Fish. Res. Board Can. 30: 187–193.

Multiple isozymes of glycerol-3-phosphate dehydrogenase (L-glycerol-3-phosphate: NAD oxidoreductase, E. C. 1.1.1.8) have been resolved by starch gel electrophoresis of extracts of muscle tissue from lake whitefish (*Coregonus clupeaformis*). The isozyme electropherograms show that three kinds of subunit, A, B, and C, are synthesized in red muscle and two of these, A and B, are also found in white muscle. In red muscle the subunits evidently combine to form catalytically active dimers of the following types: AA, AB, BB, BC, and CC. In white muscle only the AA, AB, and BB dimers were observed.

A genetic and molecular structure model is proposed for the glycerol-3-phosphate dehydrogenase (G-3-PDH) isozymes in white muscle of lake whitefish. On the basis of two alleles for A subunits and three alleles for B subunits, the model predicts a total of 18 distinct, electrophoretic G-3-PDH phenotypes for all possible AA, AB, and BB dimeric isozymes. The model has been confirmed by the results of a breeding experiment that tested the heritability of each of the five known alleles. A difference in the rate of heat inactivation of AA and BB isozymes was also interpreted as additional evidence for the unique genetic and molecular nature of the two kinds of subunits.

Surveys of natural populations of lake whitefish revealed some marked variations in the frequencies of G-3-PDH alleles in fish from different geographical areas.

The cisco (*Coregonus artedii*) also appears to have three "*b*" alleles for G-3-PDH.

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Nous avons séparé les isozymes multiples de la glycérol-3-phosphate déshydrogénase (L-glycérol-3-phosphate: NAD oxydoréductase, E.C. 1.1.1.8) par électrophorèse sur gel d'amidon d'extraits de tissu musculaire du grand corégone de lac (*Coregonus clupeaformis*). Les électrophorégrammes d'isozymes démontrent que trois sous-unités, A, B, et C, sont synthétisés dans le muscle rouge, et que deux de celles-ci, A et B, se rencontrent également dans le muscle blanc. Dans le muscle rouge, il est évident que les sous-unités se combinent pour former des dimères catalytiquement actifs des types suivants: AA, AB, BB, BC, et CC. Dans le muscle blanc, nous n'avons observé que les dimères AA, AB, et BB.

Nous proposons un modèle de structure génétique et moléculaire applicable aux isozymes de la glycérol-3-phosphate déshydrogénase (G-3-PDH) du muscle blanc du grand corégone de lac. Sur la base de deux allèles pour les sous-unités A et de trois pour les sous-unités B, le modèle prédit un total de 18 phénotypes électrophorétiques distincts de G-3-PDH pour tous les isozymes dimères possibles AA, AB, et BB. Les résultats d'un essai d'élevage vérifiant l'héritabilité de chacun des cinq allèles connus sont en accord avec le modèle. Une différence du taux d'inactivation par la chaleur des isozymes AA et BB est interprétée comme preuve additionnelle de la nature génétique et moléculaire unique des deux types de sous-unités.

Des relevés de populations naturelles de corégones démontrent des variations marquées de la fréquence des allèles de G-3-PDH chez les poissons provenant de régions différentes.

Le hareng de lac (*Coregonus artedii*) semble également posséder trois allèles "*b*" de G-3-PDH.

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STARCH gel electrophoresis of muscle extracts from lake whitefish (*Coregonus clupeaformis*) followed by an appropriate system of specific staining designed to detect zones of catalytic activity of glycerol-3-phosphate dehydrogenase (L-glycerol-3-phosphate dehydrogenase, E.C. 1.1.1.8) revealed the presence of multiple isozymes in many individual fish as well as several different phenotypes of isozymes in various populations of lake whitefish. We have developed a model to explain these observations on both a genetic and molecular basis. The model takes into account the well known dimeric nature of the catalytically active L-glycerol-3-phosphate dehydrogenase (G-3-PDH) in a wide variety of animals (Van Eys et al. 1964; Marquardt and Broesmer 1966; Broesmer and Marquardt 1966; White and Kaplan 1969). Three genetic loci, A, B, and C, with isozymes of the type AA, AB, BB, BC, and CC, are proposed to account for the different phenotypes that are observed.

In this paper we report the results of a breeding experiment that was used to investigate the heritability of multiple alleles at the A and B loci. Additional evidence from natural populations of lake whitefish is also presented that indicates both support for the genetic model as well as considerable geographic variation in the frequency of the "*a*" and "*b*" alleles.

Materials and Methods

The parental fish used in the breeding experiment were caught on their spawning grounds in Goose Creek, the outlet stream of Lake Athapapuskow, Manitoba, on October 24 and 25, 1970. A total of 29 matings were made in various combinations from 27 fish made up of 9 females and 18 males. The eggs were water hardened for a few minutes at the spawning site, then transferred to Winnipeg for incubation. The spent fish were killed, iced, and transported to the Freshwater Institute, Winnipeg, for determination of G-3-PDH phenotype.

The incubation of the fertilized eggs was conducted initially in facilities at the Department of Zoology, University of Manitoba. The eggs were contained in trays in flowing water at 4–5 C. The water was conditioned by passage through activated charcoal in an attempt to remove any possible chlorine.

On November 12, 1970, a portion of each of 20 of the individual egg lots was transferred to the Freshwater Institute. The incubation of these eggs was conducted in flowing tap water in small hatchery jars equipped with inlet pipes that extended to the bottom of each jar, thus effectively suspending the eggs in a gentle upward flowing current of water in the conventional manner. The water in this system was treated with sodium thiosulphate to neutralize any free chlorine and it was maintained by refrigeration equipment at a temperature of 1–2 C.

Hatching in the warmer conditions at 4–5 C occurred in February 1971, while those eggs incubated at the more natural temperatures of 1–2 C hatched in April 1971. After hatching was completed, the water temperature in the rearing tanks was gradually increased to 12–16 C and maintained in this range during the rearing period. The larval fish were fed a mixed diet of brine shrimp nauplii, commercial trout food, and natural plankton from local ponds.

The fish reached a useful size during August 1971 and many were killed and analyzed for G-3-PDH phenotype. The fish remaining by September 2 were killed and frozen for later G-3-PDH analysis.

We were also able to utilize some of the progeny from a previous whitefish breeding experiment (Clayton and Franzin 1970). The matings identified as 1, 2, 4, and 5 in the earlier paper are now identified as 14, 4, 18, and 1, respectively. Matings 2 and 15, not previously reported, were also from the 1968 breeding experiment. The parental fish used in matings 2 and 15 were taken from Goose Creek.

In addition to the fish utilized in the breeding experiments, we have collected lake whitefish at various times and places within Lake Athapapuskow since 1967 and these were all combined to yield the large population sample. The lake whitefish from Teenah Lake (60°18′N 133°25′W), Yukon Territory, were collected during July 1970. These fish were iced soon after death and frozen within a few days and kept for varying periods (years) at –40 C before analysis.

Cisco (*Coregonus artedii*) used in this study were from Lake Waskesiu, Prince Albert National Park, Saskatchewan, and were trapped on their normal spawning grounds near the Waskesiu River, the outlet stream, during October 1969.

The analysis of adult and juvenile lake whitefish for G-3-PDH phenotype was carried out by the same general methods as described previously (Clayton et al. 1971). The electrophoresis buffers were made up with citric acid and adjusted to *p*H 8.0 with tris (hydroxymethyl) amino methane as described previously (Clayton and Gee 1969), but with the addition of nicotinamide adenine dinucleotide (NAD) to a concentration of 100 mg/liter. Isozymes of G-3-PDH were visualized on the starch gel strips by staining at room temperature in the dark with a solution that contained the following ingredients: 5 mg NAD, 3 mg Nitro BT[1], 0.2 mg PMS[2], 3.3 mg KCN, 216 mg DL-glycerol-3-phosphate[3], and 10 ml 0.15 M Tricine[4] (*p*H 8.0, NaOH).

The heat stability of G-3-PDH isozymes was tested by heating the starch gel strips at 55 C for measured time periods after electrophoresis and before staining. The enzymatic activity was estimated quantitatively by scanning the stained strips with a Gilford Spectrophoto-

[1]Nitro BT, Dajac Laboratories, Philadelphia [2,2′-di-*p*-nitrophenyl-5,5′-diphenyl-3′-(3,3′-dimethoxy-4,4′-biphenylene)-ditetrazolium chloride].

[2]Phenazine methosulfate, Aldrich Chemical Company, Milwaukee.

[3]DL-α-glycerophosphate, Sigma Chemical Company, St. Louis.

[4]N-tris-(hydroxymethyl) methylglycine, Calbiochem, San Diego.

meter equipped with a linear transport device. The technique is described in greater detail elsewhere (Clayton et al. 1973).

Results and Discussion

Starch gel electrophoresis of extracts of white muscle from lake whitefish followed by specific staining for G-3-PDH revealed many phenotypes that individually contained variable numbers of isozymes (Fig. 1–3). We have developed a model (Fig. 4 and 5) to account for these phenotypes on a unified genetic and molecular basis. Our model was developed on the assumption that lake whitefish G-3-PDH isozymes were dimers as in other animals (Van Eys et al. 1964; Marquardt and Broesmer 1966; Broesmer and Marquardt 1966). The model also assumes two genetic loci, A and B, to be active in lake whitefish white muscle and predicts the formation of all possible types of AA, AB, and BB isozymes. A system of nomenclature based on electrophoretic mobility is used thus: A^2A^2 is faster than A^1A^1 and B^3B^3 is faster than B^2B^2, which is in turn faster than B^1B^1. The isozymes A^2A^2, A^2B^3, and B^3B^3 (Fig. 1, 3, and 5) apparently all have the same electrophoretic mobility and all attempts to separate them electrophoretically with citric acid–amine buffers in the *p*H range 6–9 (Clayton and Tretiak 1972) were unsuccessful.

In Fig. 1 we show the six phenotypes of A^2A^2 homozygotes in a sequential arrangement that clearly illustrates the progressive increase in mobility of the three B subunits. This set of phenotypes is very common in lake whitefish of central Canada. The increasing complexity of phenotypes that contain both A^1 and A^2 subunits is shown in Fig. 2 and 3. It may be seen (Fig. 1 and 2) that the A^2A^2 isozyme is less intensely stained than the BB isozymes and the A^1A^1 isozyme is very weakly stained (Fig. 3). The phenotypes are, on the whole, readily distinguishable with the exception of $A^1A^1B^3B^3$ and $A^1A^2B^3B^3$ (Fig. 3), where the weak staining of both A^1A^1 and A^1A^2 sometimes makes the classification of individual fish difficult. The conditions of the staining reaction are not entirely natural and it is likely unwise to attach any physiological significance to the relative intensity of staining of the various isozymes.

A total of 18 distinctive phenotypes are predicted (Fig. 4 and 5) on the basis of two kinds of A subunit and three kinds of B subunit. All of the possible nine phenotypes that contain B^1 subunits are illustrated in Fig. 4. All of these phenotypes have been observed except $A^1A^1B^1B^1$, $A^1A^1B^1B^2$, $A^1A^1B^1B^3$, and $A^1A^2B^1B^1$. The remaining phenotypes, composed of A^1, A^2, B^2, and B^3 subunits, as well as the subunit composition of the isozymes, are shown in Fig. 5. All of these phenotypes have been observed.

The G-3-PDH phenotypes found in laboratory reared juvenile lake whitefish are shown in Fig. 6 and 7. The weak staining of isozymes that contain A^1 subunits is even more pronounced in juvenile fish than adults. Thus, $A^1A^2B^3B^3$ juveniles (Fig. 7) display only two isozymes in comparison to the expected three isozymes that were definitely visible in adult fish (Fig. 3). While this did not cause any difficulty in the progeny analysis, the weak staining of A^1 containing isozymes in the $A^1A^2B^1B^3$ and $A^1A^2B^2B^3$ phenotypes was more troublesome and it was impossible to classify a few of the juvenile fish.

Progeny from a total of 26 individual matings were analyzed in order to determine the heritability of the various phenotypes (Tables 1 and 2). Most of the parental fish were A^2A^2 homozygotes and results from all matings between A^2A^2 homozygotes are recorded in Table 1. The matings are arranged in Table 1 in order of increasing complexity, proceeding from matings between BB homozygotes to matings between unlike heterozygotes. Only two A^1A^2 heterozygotes were included in the breeding experiment and the results of three matings with these fish are shown in Table 2.

These breeding data (Tables 1 and 2) appear to provide convincing support for our genetic and molecular model for lake whitefish white muscle G-3-PDH isozymes. There is no example of the absence of any expected phenotypes among the progeny. On the other hand, while almost 1500 progeny were analyzed, only 5 (matings 1, 14, and 25) were of unexpected phenotypes. These fish were also analyzed for malate dehydrogenase phenotype and, while the genetics of this isozyme system are yet to be elucidated, the phenotypes were also irregular with respect to this enzyme. We, therefore, suggest that these fish were most likely strays from other matings. The agreement with the model was evaluated quantitatively by comparing the observed numbers of progeny to the expected ratios via the standard x^2 test (Tables 1 and 2). In all matings except 18, 19, and 27 the probability of worse agreement to the model exceeded 0.1. These are on the whole very satisfactory results.

In mating 27 it was necessary to distinguish between A^1A^2 and A^2A^2 phenotypes and, as mentioned earlier, some of these distinctions are difficult because of the faint staining of isozymes that contain A subunits. This is likely the reason it was not possible to classify 7 of the 144 progeny that were analyzed from this mating. It is possible that inclusion of these samples could have made

a slight improvement in the probability of the results.

We have examined the G-3-PDH phenotype distribution in many lake whitefish populations and the complete results will be reported elsewhere. The phenotype distributions in two lakes, Lake Athapapuskow, Manitoba, and Teenah Lake, Yukon Territory, show substantial agreement with the distributions expected on the basis of the Hardy–Weinberg principle (Table 3). In these particular populations it was not possible to test the significance of the agreement by means of the χ^2 test because of the small expected numbers of some classes. These lake whitefish populations are typical of their regions and show the absence of the "b^1" allele in the Yukon Territory and the very rare occurrence of the "a^1" allele in central Canada.

It has already been shown (Clayton and Franzin 1970) that lake whitefish red muscle from the region of the lateral line has a complement of lactate dehydrogenase isozymes different from white muscle and it was of interest to see if additional G-3-PDH isozymes were also present in red

TABLE 1. Inheritance of BB phenotypes of glycerol-3-phosphate dehydrogenase in A^2A^2 homozygous lake whitefish (*Coregonus clupeaformis*).

Mating identification[a]		Parental phenotype	No. progeny and phenotypes							
No.	Female × Male	Female × Male	B^1B^1	B^1B^2	B^2B^2	B^1B^3	B^2B^3	B^3B^3	χ^2	p[b]
1	2060×2059	$B^1B^1 \times B^1B^1$	32					1[c]	0	1.0
2	2076×2078	$B^3B^3 \times B^3B^3$						24	0	1.0
3	3303×3308	$B^1B^1 \times B^3B^3$				22			0	1.0
4	2057×2058	$B^3B^3 \times B^1B^1$				24			0	1.0
5	3314×3311	$B^2B^2 \times B^1B^1$		48						
6	3314×3319	$B^2B^2 \times B^1B^1$		48						
	Total	$B^2B^2 \times B^1B^1$		96					0	1.0
7	3303×3309	$B^1B^1 \times B^1B^2$	29	19					2.08	0.15
8	3312×3311	$B^1B^2 \times B^1B^1$	26	22					0.33	0.6
9	3313×3311	$B^1B^3 \times B^1B^1$	21			27				
10	3313×3319	$B^1B^3 \times B^1B^1$	21			26				
	Total	$B^1B^3 \times B^1B^1$	42			53			1.27	0.3
11	3322×3327	$B^1B^2 \times B^2B^2$		21	27				0.75	0.4
12	3314×3320	$B^2B^2 \times B^1B^3$		54			41		1.78	0.2
13	3324×3327	$B^1B^3 \times B^2B^2$		21			27		0.75	0.4
14	2057×2056	$B^3B^3 \times B^1B^3$	1[c]			25		20	0.56	0.5
15	2082×2084	$B^1B^2 \times B^3B^3$				7	7		0	1.0
16	3302×3307	$B^2B^3 \times B^3B^3$					25	23	0.083	0.8
17	3312×3315	$B^1B^2 \times B^1B^2$	10	26	12				0.57	0.8
18	2062×2061	$B^1B^3 \times B^1B^3$	1			12		8		
19	3313×3320	$B^1B^3 \times B^1B^3$	25			40		31		
	Total	$B^1B^3 \times B^1B^3$	26			52		39	4.33	0.1
20	3312×3317	$B^1B^2 \times B^2B^3$		9	13	12	13			
21	3322×3326	$B^1B^2 \times B^2B^3$		27	17	24	27			
22	3323×3326	$B^1B^2 \times B^2B^3$		15	29	18	34			
	Total	$B^1B^2 \times B^2B^3$		51	59	54	74		5.26	0.15
23	3313×3318	$B^1B^3 \times B^1B^2$	19	11		20	14		3.37	0.3
24	3324×3326	$B^1B^3 \times B^2B^3$		26		28	25	17	2.92	0.4

[a]Matings are identified by numbers 1–27 and by arbitrary numbers assigned to each parental fish.
[b]Probability of worse fit to simple Mendelian expectation of ratios of numbers of progeny.
[c]These fish were likely strays and were not included in χ^2 calculation.

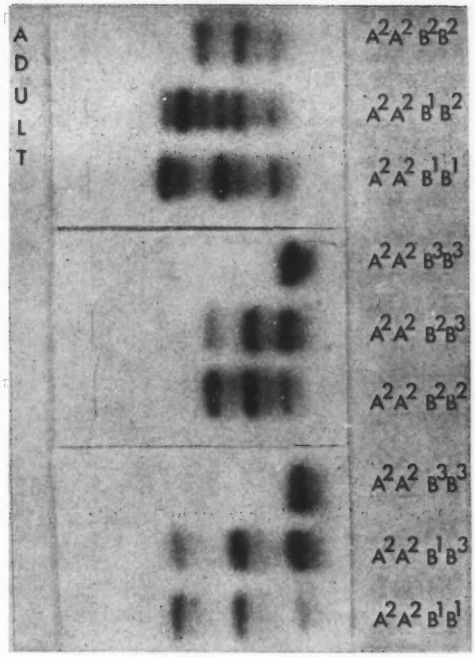

FIG. 1. Electropherograms of G-3-PDH isozymes in white muscle of A^2A^2 homozygous adult lake whitefish (*Coregonus clupeaformis*). Anode to the right.

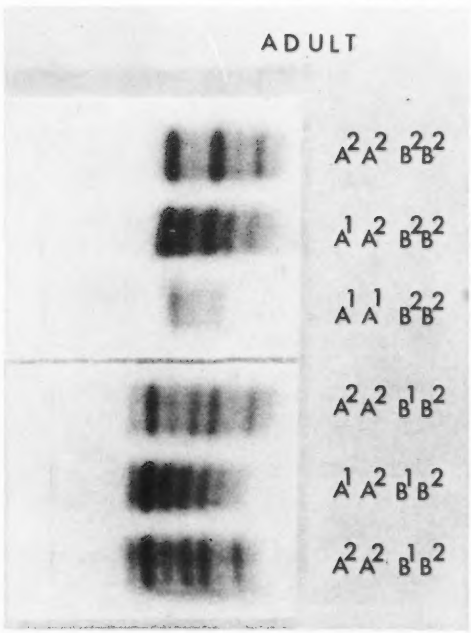

FIG. 2. Electropherograms of G-3-PDH isozymes in white muscle of adult lake whitefish. Anode to the right.

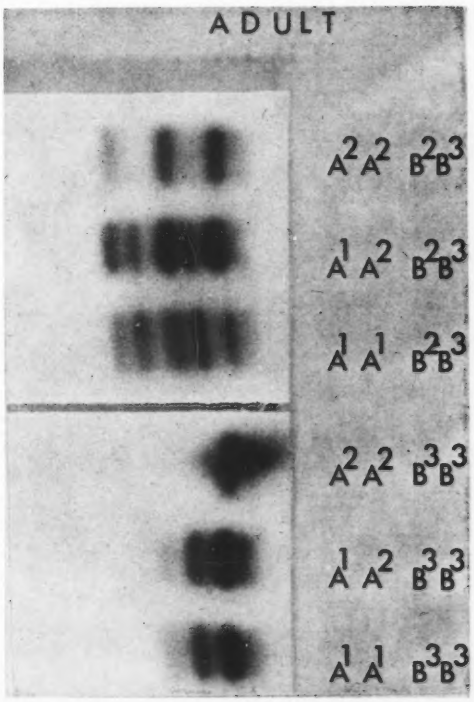

FIG. 3. Electropherograms of G-3-PDH isozymes in white muscle of adult lake whitefish. Anode to the right.

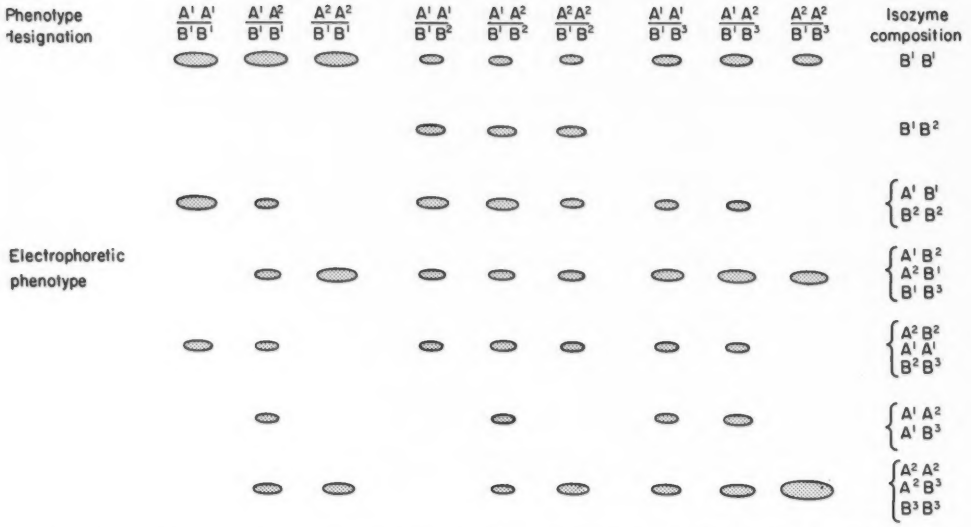

FIG. 4. Diagram of all lake whitefish white muscle G-3-PDH phenotypes that contain the B^1 subunit showing assumed subunit composition of the isozymes.

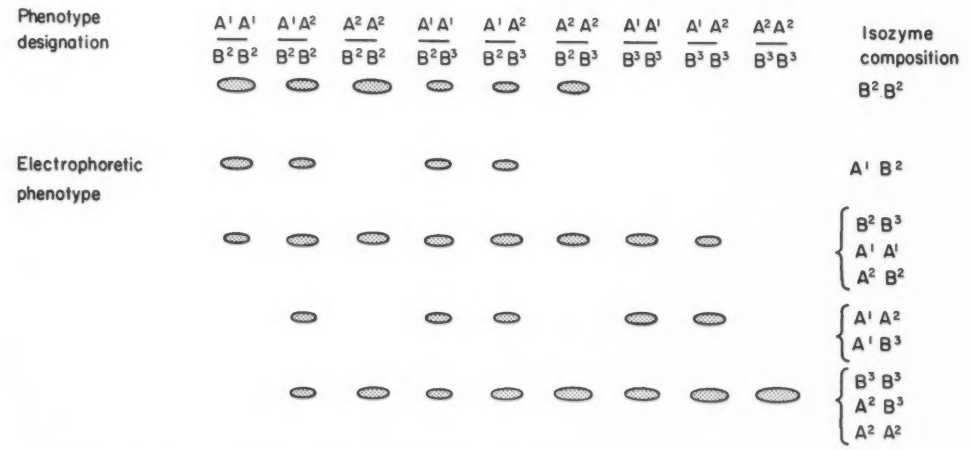

FIG. 5. Diagram of all lake whitefish white muscle G-3-PDH phenotypes that lack the B^1 subunit showing assumed subunit composition of the isozymes.

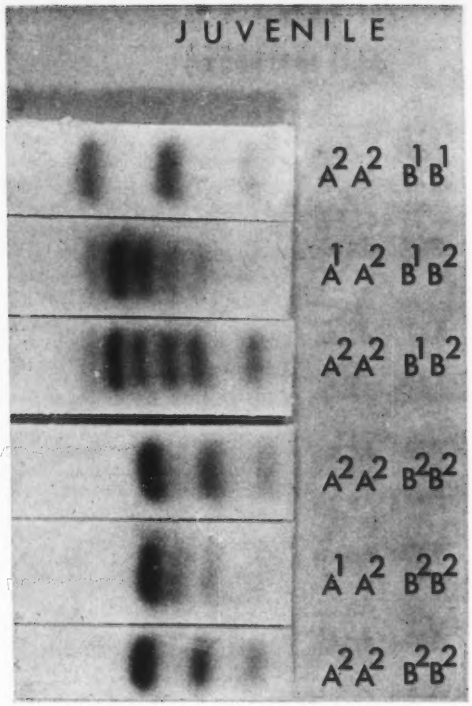

FIG. 6. Electropherograms of G-3-PDH isozymes in muscle of laboratory reared juvenile lake whitefish. Anode to the right.

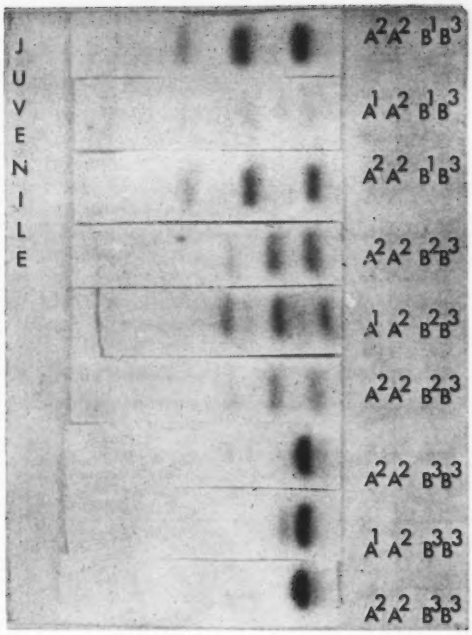

FIG. 7. Electropherograms of G-3-PDH isozymes in muscle of laboratory reared juvenile lake whitefish. Anode to the right.

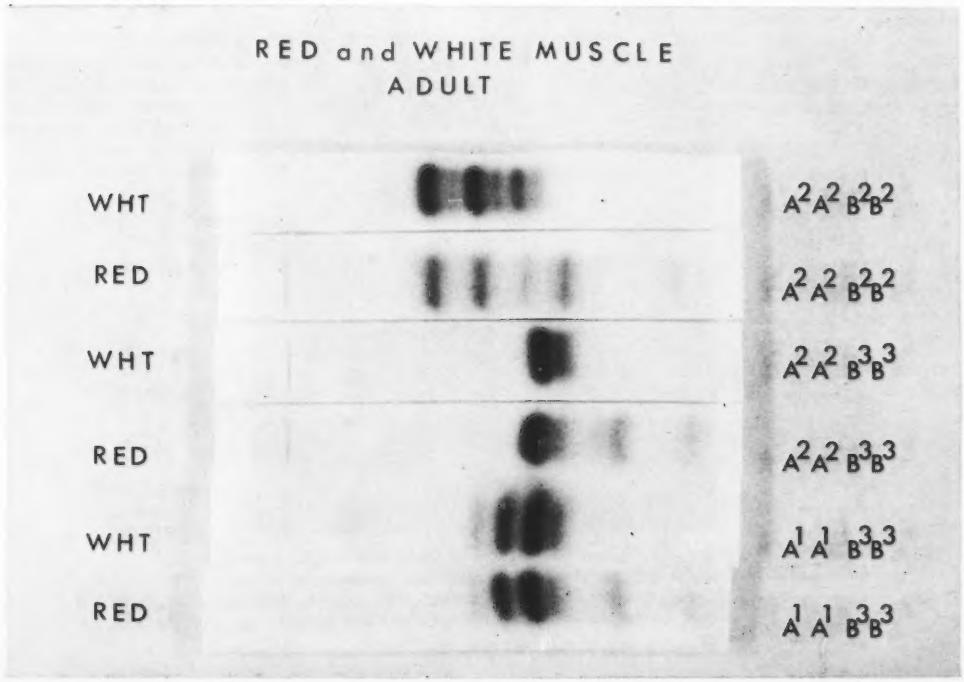

FIG. 8. Electropherograms of G-3-PDH isozymes in white and red muscle of adult lake whitefish. Anode to the right.

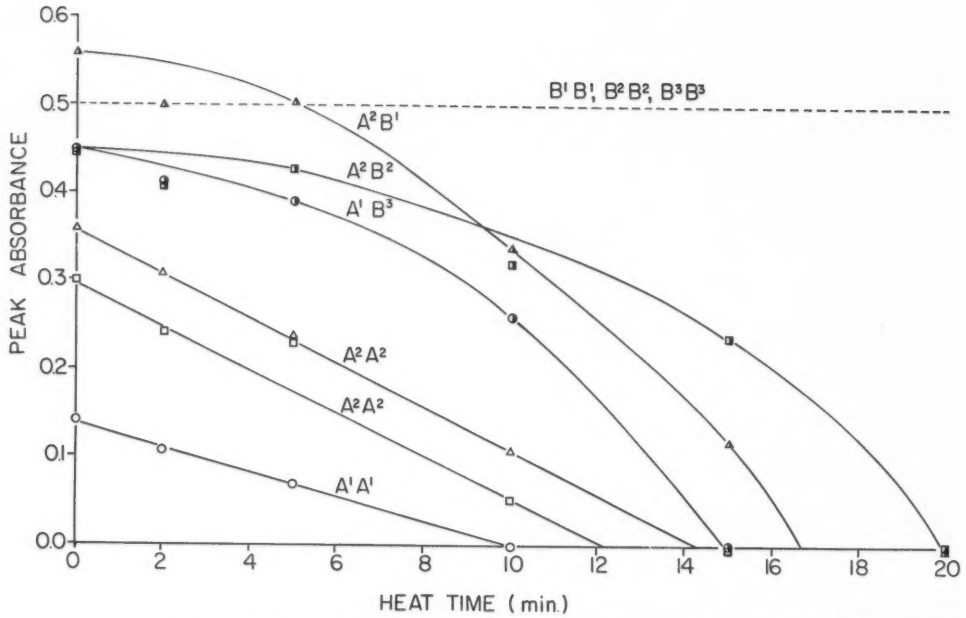

FIG. 9. Diagram showing the effect of heating at 55 C before staining on catalytic activity of G-3-PDH isozymes in white muscle of $A^2A^2B^1B^1$, $A^2A^2B^2B^2$, and $A^1A^1B^3B^3$ adult lake whitefish. Peak height = maximum absorbance of isozyme after staining.

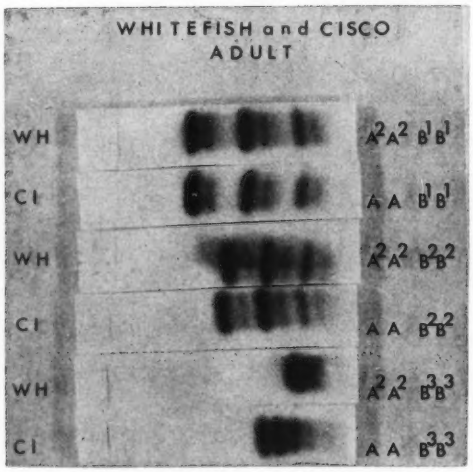

FIG. 10. Electropherograms of G-3-PDH isozymes in white muscle of lake whitefish and presumed corresponding cisco. Anode to the right. WH = lake whitefish, CI = shallow water cisco (*Coregonus artedii*).

muscle. In Fig. 8 the red and white muscle complements of G-3-PDH isozymes from three homozygous fish are compared. It may be seen that there is indeed an additional isozyme in red muscle that migrates faster anodally than the A^2A^2, A^2B^3, B^3B^3 set of isozymes. For convenience, we have designated this electrophoretically fast isozyme CC. Close inspection of Fig. 8 also shows another additional isozyme in the red muscle samples that lies approximately midway between the BB and CC isozymes in each phenotype and is evidently a BC isozyme. There seems to be no evidence of any AC isozymes.

While our own work was in its latter stages the occurrence of multiple isozymes of G-3-PDH in herring, smelt, and two trout species was reported (Engel et al. 1971). These authors have used an A,B,C system of nomenclature for the isozymes that they

TABLE 2. Inheritance of glycerol-3-phosphate dehydrogenase phenotypes in lake whitefish.

Mating identification[a]		Parental phenotype		No. progeny and phenotypes[b]						
No.	Female × Male	Female × Male		B^1B^2	B^2B^2	B^1B^3	B^2B^3	B^3B^3	χ^2	p[c]
25	3301×3304	$\frac{A^1A^2}{B^3B^3} \times \frac{A^2A^2}{B^2B^3}$	A^1A^2				10	12		
									2.38	0.5
			A^2A^2	(3)[d]			8	15		
26	3301×3305	$\frac{A^1A^2}{B^3B^3} \times \frac{A^2A^2}{B^2B^3}$	A^1A^2				9	14		
									1.50	0.7
			A^2A^2				11	14		
27	3302×3306	$\frac{A^2A^2}{B^2B^3} \times \frac{A^1A^2}{B^1B^2}$	A^1A^2	14	9	14	15			
									15.0	0.04
			A^2A^2	16	29	22	18			

[a]Matings are identified by numbers 1–27 and by arbitrary numbers assigned to each parental fish.
[b]Progeny phenotypes are entered in columns for BB and rows for AA; thus 10 A^1A^2/B^2B^3 progeny were observed from mating 25. Progeny with A^1A^1 and B^1B^1 phenotypes were neither expected nor observed and these classifications are omitted from the table.
[c]Probability of worse fit to simple Mendelian expectation of ratios of numbers of progeny.
[d]These fish were likely strays and are not included in χ^2 calculations.

TABLE 3. Distribution of glycerol-3-phosphate dehydrogenase phenotypes in natural populations of lake whitefish.[a] o = observed number. e = expected number (Hardy–Weinberg).

			B^1B^1	B^1B^2	B^2B^2	B^1B^3	B^2B^3	B^3B^3
Teenah Lake, Yukon Territory	A^1A^1	o			3		3	1
		e			1.6		4.1	2.7
	A^1A^2	o			11		25	22
		e			10.3		27.1	17.8
	A^2A^2	o			13		52	25
		e			17.1		44.8	29.4
Lake Athapapuskow, Manitoba	A^1A^2	o	–	2	–	–	–	2
		e	0.5	0.9	0.4	1.2	1.1	0.7
	A^2A^2	o	28	35	23	46	50	37
		e	21.2	40.5	19.4	53	50.7	33.2

[a]Phenotype numbers are entered in rows for AA and columns for BB; thus in Teenah Lake 3 $A^1A^1B^2B^2$ fish were found.

observed. On the basis of the phenotypes alone it is difficult to determine whether there is any correlation between the two systems of nomenclature. It does not help that lake whitefish heart extracts produced no detectable G-3-PDH isozymes on electrophoresis while Engel et al. (1971) identified isozymes containing A, B, and C subunits in extracts from hearts of both rainbow (*Salmo gairdneri*) and brown (*Salmo trutta*) trout. We have also named the most anodal isozyme CC as did Engel et al. (1971) and it is possible that there is some degree of correspondence between the CC isozymes in their study and the present work. Some doubt is, however, cast on this interpretation because we see no evidence for AC type isozymes in lake whitefish, while Engel et al. (1971) show clearly all possible dimers including AC.

We have attempted to partially characterize the AA, BB set of isozymes from white muscle by testing the relative rate of heat inactivation of the three kinds of isozyme in white muscle extracts from $A^2A^2B^1B^1$, $A^2A^2B^2B^2$, and $A^1A^1B^3B^3$ homozygous fish (Fig. 9). It is clear from this experiment that both A^1A^1 and A^2A^2 isozymes are quickly inactivated by heating while all three kinds of BB isozymes are little affected. These results should not be interpreted as no inactivation of BB, however, the data shown in Fig. 9 are calculated and the activity of the BB isozyme in each channel on the gel was arbitrarily set to a constant value by multiplying by an arbitrary factor. The activity of the AA and AB isozymes was also multiplied by the same arbitrary factor. In any case, it is clear that the G-3-PDH isozymes in lake whitefish white muscle are readily distinguished by heat stability and it seems possible that this factor could be used to establish homologies with G-3-PDH isozymes in other species. On the other hand, the coenzyme analogues deaminonicotinamide adenine dinucleotide and acetylpyridine adenine dinucleotide, which are useful for distinguishing malate dehydrogenase isozymes (Kaplan and Ciotti 1961), displayed no differential reactivity with the lake whitefish white muscle G-3-PDH isozymes.

In view of the remarkable polymorphism of G-3-PDH in lake whitefish, it was of interest to investigate the nature of the enzyme in a closely related species, the cisco (*Coregonus artedii*). A survey of a sample of 65 of these fish from Lake Waskesiu, Prince Albert National Park, Saskatchewan, revealed a set of six phenotypes of G-3-PDH in white muscle that may also evidently be attributed to three alleles at a B-type locus. The three cisco G-3-PDH homozygous phenotypes are shown beside the corresponding lake whitefish phenotypes in Fig. 10. The major difference between the two species is the electrophoretic mobility of the B^3 subunits. In whitefish the isozymes B^3B^3, A^2B^3, and A^2A^2 have very similar if not identical electrophoretic mobility and appear as a single band, while in the cisco the anticipated triplet of isozymes is clearly resolved by the electrophoretic procedure. This difference between the two species, while it is easily accommodated by our model of the genetic and molecular structure of G-3-PDH in lake whitefish, would be of little value as a taxonomic criterion because the many fish that carry only b^1 and b^2 genes would not be distinguished. Occasionally, additional sub-bands more anodal than the major isozymes occur in lake whitefish G-3-PDH electropherograms and these are quite pronounced in Fig. 10. These sub-bands do not seem to interfere with our discrimination of the various phenotypes and we have not made any attempt to identify them. It seems quite likely that they occur when the protein components of the major isozymes bind some unknown negatively charged small molecule such as a phosphate or polyphosphate during electrophoresis.

We conclude from the results of this study that our model of two loci, A and B, with two "*a*" alleles and three "*b*" alleles, adequately describes the genetics of G-3-PDH in white muscle of lake whitefish. Lake whitefish white muscle G-3-PDH phenotypes as revealed by starch gel electrophoresis are readily accounted for by the random combination of subunits into all the possible dimers. The model predicts a total of 18 genotypes and an equal number of distinguishable phenotypes. As yet, only 14 phenotypes have been observed because no population of lake whitefish has been discovered that has both the "a^1" and "b^1" genes in moderate abundance. The AA and BB isozymes are distinguished by the higher rate of thermal inactivation of AA isozymes as compared to BB isozymes as well as by their electrophoretic mobility. It seems likely that red muscle contains an additional genetic locus (C) for G-3-PDH, and both CC and BC isozymes, as well as AA, AB, and BB isozymes, are produced in this tissue.

A preliminary electrophoretic examination of some cisco also showed these animals to have two G-3-PDH loci active in white muscle producing AA, AB, BB isozymes. These cisco also appear to have three "*b*" alleles for G-3-PDH, like lake whitefish.

Acknowledgments

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assisted in the electrophoretic analyses. R. E. K. Harris, W. A. MacDonald, and J. Cook assisted with the incubation and rearing of the fish as well as with electrophoretic analyses. Financial support for the project in the form of National Research Council grants to C. C. Lindsey is also gratefully acknowledged.

BROESMER, R. W., AND R. R. MARQUARDT. 1966. Insect extramitochondrial glycerophosphate dehydrogenase. II. Enzymic properties and amino acid composition of the enzyme from honey bee (*Apis mellifera*) thoraces. Biochim. Biophys. Acta 128: 464–473.

CLAYTON, J. W., AND W. G. FRANZIN. 1970. Genetics of multiple lactate dehydrogenase isozymes in muscle tissue of lake whitefish (*Coregonus clupeaformis*). J. Fish. Res. Board Can. 27: 1115–1121.

CLAYTON, J. W., AND J. H. GEE. 1969. Lactate dehydrogenase isozymes in longnose and blacknose dace (*Rhinichthys cataractae* and *R. atratulus*) and their hybrid. J. Fish. Res. Board Can. 26: 3049–3051.

CLAYTON, J. W., R. E. K. HARRIS, AND D. N. TRETIAK. 1973. Malate dehydrogenase isozymes in walleye and sauger (*Stizostedion vitreum vitreum* and *S. canadense*). J. Fish. Res. Board Can. (In press)

CLAYTON, J. W., AND D. N. TRETIAK. 1972. Amine-citrate buffers for *p*H control in starch gel electrophoresis. J. Fish. Res. Board Can. 29: 1169–1172.

CLAYTON, J. W., D. N. TRETIAK, AND A. H. KOOYMAN. 1971. Genetics of multiple malate dehydrogenase isozymes in skeletal muscle of walleye (*Stizostedion vitreum vitreum*). J. Fish. Res. Board Can. 28: 1005–1008.

ENGEL, W., J. SCHMIDTKE, AND U. WOLF. 1971. Genetic variation of α-glycerophosphate dehydrogenase isoenzymes in clupeoid and salmonoid fish. Experientia (Basel) 27: 1489–1491.

KAPLAN, N. O., AND M. M. CIOTTI. 1961. Evolution and differentiation of dehydrogenases. Ann. N.Y. Acad. Sci. 94: 701–720.

MARQUARDT, R. R., AND R. W. BROESMER. 1966. Insect extramitochondrial glycerophosphate dehydrogenase. I. Crystallization and physical properties of the enzyme from honey bee (*Apis mellifera*) thoraces. Biochim. Biophys. Acta 128: 454–463.

VAN EYS, J., J. JUDD, J. FORD, AND W. B. WOMACK. 1964. On the chemistry of rabbit muscle α-glycerophosphate dehydrogenase. Biochemistry 3: 1755–1763.

WHITE, H. B. III,. AND N. O. KAPLAN. 1969. Purification and properties of two types of diphosphopyridine nucleotide linked glycerol-3-phosphate dehydrogenases from chicken breast muscle and chicken liver. J. Biol. Chem. 244: 6031–6039.

An Evaluation of RNA–DNA Ratio as a Measure of Long-Term Growth in Fish Populations

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HAINES, T. A. 1973. An evaluation of RNA–DNA ratio as a measure of long-term growth in fish populations. J. Fish. Res. Board Can. 30: 195–199.

The value of RNA–DNA ratio as a measure of long-term growth of fish populations under semi-natural conditions and when subjected to environmental manipulations was determined. Populations of carp and smallmouth bass of known age distribution were established in artificial ponds maintained at two fertility levels. After 15 months, population growth rates (as percent increase in weight) and RNA–DNA ratios of muscle tissue from selected fish were measured. Each species exhibited a range of population growth rates. The relation between population growth rate and individual fish RNA–DNA ratio for each species was significant. When reproduction occurred, the relation was not significant unless young-of-the-year fish were excluded from population growth rate calculations. Age of fish was also found to have an important effect on RNA–DNA ratio, with the ratio being higher in younger fish.

RNA–DNA ratio can be a reliable indicator of long-term population growth in fish when population age structure is known and recruitment is controlled. The method has potential for use in detecting response to environmental changes before growth rate changes become severe.

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L'auteur a entrepris cette expérience dans le but d'évaluer le rapport RNA–DNA comme mesure de la croissance à long terme des populations de poissons dans des conditions semi-naturelles et dans un environnement soumis à des manipulations. Il a établi, dans une série d'étangs artificiels, des populations de deux espèces (carpe et achigan à petite bouche) d'âge connu. Il y a assuré deux paliers de fertilité par additions régulières d'azote et de phosphore solubles. Au bout de 15 mois, les poissons ont été tués, le rythme de croissance des populations (en pourcentage d'augmentation de poids) a été déterminé, et le rapport RNA–DNA du tissu musculaire de poissons choisis a été mesuré. La croissance des populations de chaque espèce varie en réponse aux conditions auxquelles elles ont été soumises. L'auteur analyse la relation entre le rythme de croissance de la population et le rapport RNA–DNA de chaque poisson des deux espèces étudiées.

Les résultats démontrent une relation significative (au niveau de 5%) entre le rapport RNA–DNA de poissons individuels et le rythme de croissance à long terme de la population. Au moment de la reproduction, cette relation ne tient plus, si on n'exclut pas des calculs les jeunes de l'année. L'âge du poisson a également un effet notable sur le rapport RNA–DNA, sa valeur étant plus élevée chez les jeunes poissons.

L'auteur conclut en affirmant que le rapport RNA–DNA est un indicateur fiable de la croissance à long terme des populations de poissons, pourvu que l'âge des individus soit connu et le recrutement contrôlé. De plus, cette méthode offre des possibilités lorsqu'il s'agit de détecter la réponse des populations aux variations du milieu, et ce, avant que le rythme de croissance ne change de façon trop prononcée.

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IT has been proposed (Bulow 1970) that the ratio of ribonucleic acid (RNA) concentration to deoxyribonucleic acid (DNA) concentration in body tissues could be used to measure recent growth in fish. Since growth in fish is accomplished primarily by protein synthesis, a measure of the organizer of protein synthesis (RNA) could be used as a measure of the growth rate of the organism. The ratio of RNA to DNA is generally considered a more accurate index than RNA concentration

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alone, since this ratio is not affected by cell number or size in the tissue sampled (Hotchkiss 1955).

This experiment tests the relation between RNA–DNA ratio and the known long-term growth rate of two species of fish in a semi-natural environment, and when subjected to environmental manipulations.

Materials and Methods

An experiment was designed (Haines 1973) to test the effects of nutrient enrichment and interspecific competition on the growth rate of populations of a game fish (smallmouth bass) and a rough fish (carp). Selected fish from these populations were used to examine the relation between RNA–DNA ratios in individual fish and population growth rates over a range of conditions.

Six ponds, 7.2 × 7.2 × 1.8 m deep, were used. The ponds had concrete walls and gravel bottoms. For purposes of replication, the ponds were divided in half by use of polyethylene film supported with nylon netting. The ponds were filled to a depth of 1.2 m with untreated well water. The water had a *p*H of 8.3 and a total alkalinity of 200 mg/liter as $CaCo_3$. Two fertility levels were established. Low-fertility ponds were untreated while high-fertility ponds received biweekly (i.e. once every 2 weeks) additions of nitrogen and phosphorus. Approximately 250 g of urea (45% N) and 110-g superphosphate (20% P) were dissolved in water and broadcast over each pond for each application.

Smallmouth bass used in the experiment were obtained from a private hatchery in Pennsylvania; carp were seined from canals at the Crane Creek National Wildlife Refuge on Lake Erie near Toledo, Ohio. Bass were of known age and carp were aged by the scale method, with any questionable fish being rejected. Estimates of naturally occurring standing crop biomass and age structure for smallmouth bass and carp populations were obtained from the literature (Buck and Thoits 1970; Carlander 1955; Coble 1971; Mraz and Cooper 1957; Surber and Everhart 1950). I selected 20 kg/ha as a reasonable biomass for smallmouth bass, and 70 kg/ha for carp. Only age 0 and 1 fish were used because of availability and the small size of the ponds. For this experiment, each pond (0.0027 ha area) which was to receive bass was stocked with 10 age 0 fish (mean weight 2.0 g) and two age I fish (mean weight 13.0 g). Each carp population consisted of 18 age 0 fish (mean weight 4.5 g) and four age I fish (mean weight 25.8 g).

Three fish species combinations were used: bass only, carp only, and bass and carp combined. The same biomass and age structure was used for each species population regardless of whether the species was alone or in combination with the other species. Replicates (i.e. two ponds each) of each of the three species combinations were established at each of the two fertility levels (low and high) used. These combinations gave four experimental conditions (each replicated) for each species:

1, bass only, low fertility
2, bass with carp present, low fertility
3, bass only, high fertility
4, bass with carp present, high fertility
5, carp only, low fertility
6, carp with bass present, low fertility
7, carp only, high fertility
8, carp with bass present, high fertility.

The fish populations were maintained for 15 months. At the end of this period the ponds were drained and all fish recovered. Fish from each pond were sorted by species, washed, drained briefly, and weighed to the nearest gram. Large fish were weighed individually; small fish were counted and weighed in groups. Although spawning was intended to be eliminated for this experiment, carp spawned successfully in the high fertility ponds.

Population growth rate, expressed as percent increase in weight of all fish combined over the term of the experiment, was calculated for each species and experimental condition. Where they occurred, age 0 carp were counted and weighed but not included in population growth rate calculations for this experiment.

Fish were selected from each population for RNA–DNA analysis. Two distinct size categories of fish were usually present, corresponding to the two age-classes originally introduced (now ages I and II). Where they occurred, age 0 carp constituted a third size category. Four fish were randomly selected from each size category in each population. Age 0 carp were not included in the main analysis, but a small number were subjected to analysis to obtain some idea of the RNA–DNA ratio in this age-class.

A scale sample was taken from each fish, then an incision was made alongside the dorsal fin, the skin was peeled down, and approximately 2 g of muscle tissue was removed from the midsection of the fish, above the backbone and below the dorsal fin. The tissue sample was placed in a glass vial and frozen immediately at –20 C. This location was selected for convenience of sampling. Use of a standard location for tissue sampling for all fish should eliminate any variation which might result from differences in allometric growth.

Ages of the fish were confirmed by the scale method, and two fish each of age I and age II were selected from each group for RNA–DNA analysis, except that in two cases only one age II bass survived to the end of the experiment.

The RNA–DNA analysis procedure used was that described by Bulow (1970), except that a Beckman model DK-2A spectrophotometer (Beckman Instrument Co., Fullerton, Calif.) was used to measure optical densities, and 50 mg of dry, fat-free muscle tissue was used in the nucleic acid extraction procedure. Presence of chromogenic interferences in the orcinol reaction was checked by dividing five randomly selected tissue samples in half, and including a cold trichloroacetic acid (TCA) extraction after defatting but before drying for one set of the samples. The nucleic acid extracts were then analyzed similarly. Comparison of the results indicated that no such interferences were present and

the cold TCA extraction was dropped. Two determinations were made for each parameter for each tissue sample (representing one fish), and variation between these determinations was small, never exceeding 10%. The mean of the two determinations was used as the best estimate of the actual value. The ratio of RNA to DNA was computed for each tissue sample using the mean values of RNA and DNA concentrations.

RNA–DNA ratios of individual fish from a given experimental condition were plotted against mean growth rate of populations from which the fish were selected. This was done for all experimental conditions with both species of fish and both age-classes. Regression lines were computed by the least squares method. The relation between individual RNA–DNA ratios and population growth rate was determined using an analysis of variance with model I regression (Sokal and Rolf 1969).

Results

The fish populations responded to the various environmental conditions with a range of growth rates. The relation between environmental condition and population growth rate for each species is discussed elsewhere (Haines 1973).

The ranges of the RNA and DNA concentrations found in this study and those for other fish species are given in Table 1. The values for bluegill were calculated from Bulow (1971) according to formulae given. The generally good agreement between species suggests that the values obtained in this experiment are reliable.

The results of the regression of RNA–DNA ratio on population growth rate are shown in Fig. 1 and 2. The analysis of variance with regression tables is given in Table 2. The error mean square was used as the denominator in determining all F values. Regression was significant at the 5% level in all cases; deviation from regression was nonsignificant at the 5% level for all except age I carp.

Age I fish always had larger RNA–DNA ratios than age II fish. Although this response was not subjected to covariance analysis, the 95% confidence belts of the regression lines were determined and rarely overlap (Fig. 1 and 2).

If age 0 carp which resulted from successful spawning in high fertility ponds were included in population growth rate calculations for these ponds, deviation from regression was significant for both age-groups of carp. RNA–DNA ratios were determined for 10 age 0 carp and were the highest of any age-group, as expected, averaging 22.31 (range 21.10–24.30).

Discussion

The results indicate that RNA–DNA ratio is a good indicator of fish population growth rate under the conditions employed. Regression was highly significant ($P < 0.01$) in all cases, indicating that there is a relation between RNA–DNA ratio and population growth rate. Deviation from regression was very low for smallmouth bass populations, the probability of occurrence never exceeding 0.5. Carp populations, however, had much higher deviation from regression (age I $P = 0.02$, age II $P = 0.10$). This may be a result of the successful spawning of carp in the high fertility ponds. Although young-of-the-year carp were excluded from population growth rate calculations, the adult carp may have used in reproduction energy that could have been used for body tissue synthesis. Variation in reproduction could then result in variation in growth rate of the older fish and may account for increased deviation from regression observed in these populations. If young-

TABLE 1. Comparison of RNA and DNA concentrations in muscle tissue of various species of fish. Concentrations are expressed as μg/100 mg tissue (wet weight).

Species	RNA	DNA	Reference
Carp	266–1039	24–79	Present study
Smallmouth bass	470–700	19–41	" "
Sockeye salmon	47–70	21–33	Creelman and Tomlinson 1959
Sockeye salmon	100	2.5	Bluhm and Tarr 1957
Chinook salmon	142	1.4	" "
Pink salmon	42	0.2	" "
Coho salmon	123	1.5	" "
Ling cod	47.3	1.0	" "
Starry flounder	48.0	2.0	" "
Pacific herring	10.4–26.5	1.87–3.51	Tarr 1958
Bluegill	385.0–524.5	18.0–30.9	Bulow 1971

of-the-year carp were included in carp population growth rate computations, deviation from regression was highly significant ($P < 0.01$).

RNA–DNA ratio reflects only the rate of protein synthesis in individual fish sampled. Population

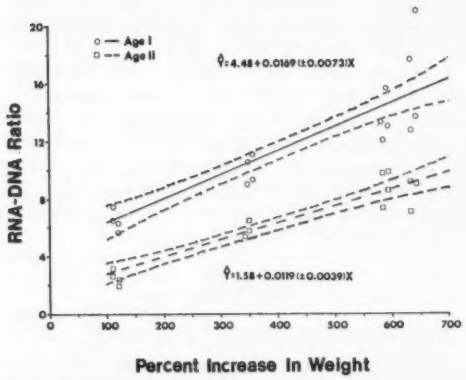

FIG. 1. Regression of individual RNA–DNA ratio against population growth rate for age I and age II smallmouth bass. The 95% confidence belts for the regression lines are shown (broken rules), the regression equations are given, and the 95% confidence limits for the slope are indicated (in parentheses).

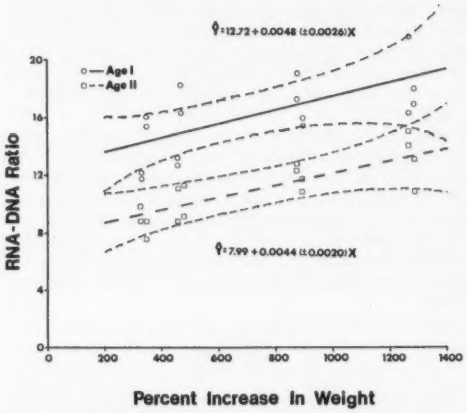

FIG. 2. Regression of individual RNA–DNA ratio against population growth rate for age I and age II carp. The 95% confidence belts for the regression lines are shown (broken rules), the regression equations are given, and the 95% confidence limits for the slope are indicated (in parentheses).

TABLE 2. Analysis of variance with regression tables.

Source of variation	df	Sums of squares	Mean square	F	$F_{0.95}$
Smallmouth bass, age I					
Regression	1	206.03	206.03	31.08	7.57
Deviation from regression	6	15.99	2.67	0.40	4.65
Error	8	53.07	6.63		
Total	15	275.09			
Smallmouth bass, age II					
Regression	1	101.50	101.50	42.65	7.57
Deviation from regression	6	7.94	1.32	0.56	4.65
Error	6	14.28	2.38		
Total	13	123.72			
Carp, age I					
Regression	1	49.83	49.83	19.70	7.57
Deviation from regression	6	101.13	16.86	6.66	4.65
Error	8	20.23	2.53		
Total	15	171.19			
Carp, age II					
Regression	1	42.22	42.22	27.24	7.57
Deviation from regression	6	32.16	5.36	3.46	4.65
Error	8	12.40	1.55		
Total	15	86.78			

growth rate changes which result from immigration, reproduction, emigration, mortality, or reproductive failure would not directly affect RNA–DNA ratio, although compensatory changes in individual growth rates which may occur in response to the above changes would affect RNA–DNA ratio. In this study, migrations could not occur and reproduction was absent (except for carp in high fertility ponds). Mortalities did occur but were not excessive, and were more than compensated for by growth of surviving fish in all cases. The major response of fish populations to environmental manipulation was variation in growth rate, and this was reflected in RNA–DNA ratios of individual fish. Fish have a highly plastic growth rate and often respond to environmental changes by changes in growth rate (Backiel and Le Cren 1967). If this is the major mechanism of response, then RNA–DNA ratio could be a useful tool in detecting favorable or unfavorable environmental conditions. Since changes in RNA should precede changes in protein synthesis rate, this method may be useful in detecting environmental change before a large change in fish growth rate occurs.

Age of fish has an important effect on RNA–DNA ratio. In this study, age I fish consistently had higher ratios than age II fish. Since young fish normally have higher growth rates than older fish (Brown 1957), this is to be expected. In order to apply this technique to natural populations, some knowledge of age distribution of the population would be necessary, and fish of various ages should be sampled.

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BACKIEL, T., AND E. LE CREN. 1967. Some density relationships for fish population parameters, p. 261–294. *In* S. Gerking [ed.] The biological basis of freshwater fish production. John Wiley & Sons, New York, N.Y.

BLUHM, H., AND H. L. A. TARR. 1957. Marine fish muscle nucleic acids. Can. J. Biochem. Physiol. 35: 767–769.

BROWN, M. 1957. Experimental studies on growth, p. 361–400. *In* M. Brown [ed.] The physiology of fishes, Vol. 1. Academic Press, Inc., New York, N.Y.

BUCK, D., AND C. THOITS III. 1970. Dynamics of one-species populations of fishes in ponds subjected to cropping and additional stocking. Ill. Natur. Hist. Surv. Bull. 30: 69–165.

BULOW, F. 1970. RNA–DNA ratios as indicators of recent growth rates of a fish. J. Fish. Res. Board Can. 27: 2343–2349.

1971. Selection of suitable tissues for use in the RNA–DNA ratio technique of assessing recent growth rate of a fish. Iowa State J. Sci. 46: 71–78.

CARLANDER, K. 1955. The standing crop of fish in lakes. J. Fish. Res. Board Can. 12: 543–570.

COBLE, D. 1971. Effects of fin clipping and other factors on survival and growth of smallmouth bass. Trans. Amer. Fish Soc. 100: 460–473.

CREELMAN, V., AND N. TOMLINSON. 1959. Biochemical studies of sockeye salmon during spawning migration. VI. Ribonucleic acid and deoxyribonucleic acid. J. Fish. Res. Board Can. 16: 421–428.

HAINES, T. 1973. Effects of nutrient enrichment and a rough fish population (carp) on a game fish population (small-mouth bass). Trans. Amer. Fish. Soc. (In press)

HOTCHKISS, R. 1955. The biological role of the deoxypentose nucleic acids, p. 435–473. *In* E. Chargaff and J. Davidson [ed.] The nucleic acids, chemistry and biology, Vol. 2. Academic Press, Inc, New York, N.Y.

MRAZ, D., AND E. L. COOPER. 1957. Natural reproduction and survival of carp in small ponds. J. Wildlife Manage. 21: 66–69.

SOKAL, R., AND F. ROLF. 1969. Biometry. W. H. Freeman and Co., San Francisco, Calif. 776 p.

SURBER, E. W., AND W. H. EVERHART. 1950. Biological effects of nigrosine used for control of weeds in hatchery ponds. Progr. Fish-Cult. 12: 135–140.

TARR, H. L. A. 1958. Biochemistry of fishes. Annu. Rev. Biochem. 27: 223–244.

Effect of Simulated Meal Size on Gastric Acid and Pepsin Secretory Rates in Bluegill (*Lepomis macrochirus*)[1]

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NORRIS, J. S., D. O. NORRIS, AND J. T. WINDELL. 1973. Effect of simulated meal size on gastric acid and pepsin secretory rates in bluegill (*Lepomis macrochirus*). J. Fish. Res. Board Can. 30: 201–204.

Rates of gastric acid and pepsin secretion into the gastric juice were examined in terms of enzymatic activity (measured spectrophotometrically as tyrosine released from hemoglobin substrate) and total gastric enzyme secretion (calculated from secretion of ^{14}C-labelled protein) at various intervals up to 9 hr following introduction into the stomach of one or two glass beads representing approximately 0.62 and 1.26% of the bluegill (*Lepomis macrochirus*) body weight, respectively. Observations of enzymatic activity and concentration suggested that gastric secretory reserves would have been depleted 6 hr after consuming a meal equalling 1.26% of the fish body weight. Decreased acidity of gastric juice 5 hr after ingesting two glass beads supported this interpretation. The importance of these data to efficiency and growth studies is discussed.

NORRIS, J. S., D. O. NORRIS, AND J. T. WINDELL. 1973. Effect of simulated meal size on gastric acid and pepsin secretory rates in bluegill (*Lepomis macrochirus*). J. Fish. Res. Board Can. 30: 201–204.

Nous avons mesuré, chez des crapets à oreilles bleues, les taux de sécrétion de l'acide gastrique et de la pepsine dans le suc gastrique, en termes d'activité enzymatique (mesurée au spectrophotomètre sous forme de tyrosine libérée du substrat d'hémoglobine) et de sécrétion totale de l'enzyme gastrique (calculée à partir d'une protéine marquée au ^{14}C). Ces mesures ont été effectuées à divers intervalles, allant jusqu'à 9 heures, après introduction dans l'estomac du poisson d'une ou deux perles de verre représentant environ 0.62 et 1.26% du poids du poisson. L'activité et les concentrations enzymatiques suggèrent que les réserves de sécrétion gastrique auraient été épuisées 6 heures après un repas équivalent à 1.26% du poids du corps. Une diminution de l'acidité du suc gastrique 5 heures après ingestion de deux perles de verre corroborent cette interprétation. Nous discutons de l'importance de ces résultats dans des études d'efficacité de conversion des aliments et de croissance.

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SULLIVAN (1907) demonstrated gastric acidity in elasmobranch fishes, which was later attributed to the secretion of free hydrochloric acid (Dobreff 1927). Dobreff (1927) observed that gastric acid secretion continued at a low level in starved elasmobranchs until death, indicating ability to secrete acid in the absence of food. He observed a slow increase in gastric acidity over a 4-day period following feeding of fasted elasmobranchs. The slow rise in acidity was attributed to absorption of secreted acid by the food mass. Dobreff's speculation was supported by measurements of *p*H at and just beneath the surface of the digesting food mass in the piscivorous pike, *Esox* (Vonk and Mennega 1938; Vonk 1939). Acidity was found to be greater at the surface of the food mass (*p*H 2.4–3.6) than 3 mm below the surface (*p*H 4.7). In general, measurements of stomach acidity in fishes have demonstrated a lower *p*H for full stomachs than for empty stomachs (see Barrington 1957).

Methodology applicable to examination of gastric digestion or evacuation or both in fishes has been extensively reviewed (Windell 1966, 1967, 1968; Davis and Warren 1968). These methods measure rate of removal of material from the stomach but not enzymatic activity or the secre-

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tion of mucus and acid. Phillips (1969) reviewed the literature on digestive capacity in terms of gastric acid and enzyme secretion as influenced by temperature, but did not discuss meal size as a variable. Windell et al. (1969) reported the effect of various meal sizes on the rate of gastric evacuation in rainbow trout (*Salmo gairdneri*). They suggested that feeding in excess of 1.1% of the fish body weight (expressed as dry matter per total live weight) resulted in the expulsion of food from the stomach before the gastric phase of digestion was completed. Presumably, this would be true only if the fish were not able to increase gastric secretion in proportion to meal size.

The objective of the present study was to examine rates of gastric acid and pepsin secretion together with measurement of hydrolytic activity of secreted pepsin in response to the size of meal ingested by bluegill sunfish (*Lepomis macrochirus*).

Materials and Methods

Bluegill sunfish were collected locally by seining and were acclimated to 20–22 C in the laboratory.

Experiments were carried out in a large Biotronette Mark III Environmental Chamber. The air exhaustion system was modified so that $^{14}CO_2$ could be removed from the effluent air by a chemical trap (BaOH). Tops were cut from four 38-liter plastic disposable containers and 34 liters of charcoal-filtered tap water was placed in each. Six bluegill sunfish (6–18 g, 71–103 mm) were introduced into each container for 3 days prior to the experiment. The water in these containers was aerated continuously. Duration of fluorescent lighting was controlled automatically to simulate a 12-hr light/dark cycle. At the time of the experiment any unhealthy fish were eliminated.

A preliminary experiment suggested that use of glass beads to simulate food would facilitate removal of a uniform quantity of gastric secretion (adhering to the surface of the bead) from each test animal at sacrifice. Gastric juice containing ^{14}C-labelled protein was mixed with glass beads. The beads were analyzed individually and in duplicate to determine the activity of enzyme and radioactivity of protein adhering to each bead. Statistical analysis of these data (student's *t* test) indicated there were no significant differences among beads in terms of enzymatic activity of radioactivity. Uptake was linear over a five fold range of dilution.

A test meal consisted of one or two small glass beads (diameter, 4 mm; volume, 33.4 mm^3; surface area, 50.2 mm^2). Each fish was hand-held and one glass bead at a time was placed gently into the esophagus. A polished glass rod was used to move the bead to the point where swallowing occurred reflexively. Hand-fed fish were returned to the environmental chamber until sacrificed at predetermined intervals (5, 10, 15, 20, 30, 45, 60 min, 2, 3, 5, 7, or 9 hr after feeding). Sacrifice intervals were based upon known digestive rates for bluegill sunfish maintained at 20–22 C (Windell 1966, 1968). Mean weight and length of fish fed one bead were 10.0 g (6.0–18.5 g) and 83 mm (71–103 mm), respectively. Fish fed two beads averaged 9.8 g in weight (6.6–16.3 g) and 84.1 mm in length (71–94 mm).

Glass beads were removed immediately from the stomach of each sacrificed fish by autopsy (Windell 1966), placed in 15-ml polyethylene centrifuge tubes (prewashed with 0.1-N HCl), and frozen at –15 C for later analysis of enzymatic activity.

The *p*H of gastric secretions present at autopsy was determined visually by dropping a sample of gastric juice directly onto *p*H-indicator paper.

Radioisotopic determination of total gastric protein secretion was performed by injecting bluegill sunfish with a mixture of DL-alanine-1-^{14}C and D-glycine-1-^{14}C (0.5 $\mu c/g$ fish). Fish were fed mealworms (*Tenebrio molitor*) ad libitum and fasted for 72 hr to completely empty their stomachs of food. Fasted fish were force-fed one or two glass beads and sacrificed at predetermined intervals. The beads recovered at autopsy were frozen until analyzed for radioactivity and pepsin content.

Enzymatic activity was examined using a modification of a technique described by Bergmeyer (1965). A 1% hemoglobin solution was incubated at 37 C until equilibrated. A sample of the equilibrated solution (2.5 ml) was placed into a 15-ml centrifuge tube with the glass bead(s) recovered from the stomach at autopsy. Each tube was incubated at 37 C for 10 min. Enzymatic hydrolysis of the hemoglobin was stopped by the addition of 5 ml of 5% trichloracetic acid (TCA). All tubes were centrifuged at $200 \times g$ for 15 min. Optical density of the clear supernatant (containing hydrolysis products) was determined at 280 $m\mu$ in a spectrophotometer. The hemoglobin pellet remaining after centrifugation and the gastric juice adhering to the bead(s) were hydrolyzed for 24 hr at 50 C following the addition of 2 ml of 0.1-N sodium hydroxide solution to each centrifuge tube. Each TCA-soluble supernatant (having been measured for optical density) was returned to its original tube. The resulting solution was shaken to insure thorough mixing and was decolorized by the addition of 0.5 ml of 30% hydrogen peroxide. After incubating for 24 hr or more, a 0.5 ml sample was removed from each tube and placed in a scintillation vial containing a 15-ml solution of toluene, 2,5-diphenyloxazole and BBS-3 (Beckman Instruments) in a ratio of 200:1:20. Radioactivity of each vial was determined by liquid scintillation counting at 60–70% efficiency for a sufficient period of time to reduce the standard deviation of the counts per minute to 0.2%.

The percentage of ^{14}C-labelling that could be assigned to protein in the gastric juice was obtained following digestion of the protein components with papain (Glass 1968). The digest was dialyzed twice against distilled water. Combined distilled water fractions (containing hydrolysis products) and the undigested material remaining in the dialysis tubing was brought to constant volume, diluted with an equal volume of 2-N sodium hydroxide, and incubated for 24 hr at 50 C. Each solution was dialyzed against 3-M sucrose until approximately one-half the original volume remained. The volume of the solution containing hydrolysis products was

adjusted with the addition of distilled water to equal that containing the undigested material. The solutions were decolorized and the radioactivity of 0.5-ml samples determined as above.

Results

The *p*H of the gastric juice decreased from approximately 5 to between *p*H 1 and 2 after ingestion of one or two glass beads (Fig. 1). Gastric *p*H remained low in fish fed one glass bead but rose markedly after 3 hr in fish fed two glass beads (to *p*H 5).

Total enzymatic activity (OD) measured during the first 5 hr of digestion was greater in fish fed two beads (Fig. 2 A) than in those fed a single bead (Fig. 2 B). Maximum enzymatic activity was recorded at 1 hr following ingestion for fish fed one bead and at 5 hr for fish fed two beads. However, after 5 hr the level of enzymatic activity of pepsin adhering to two beads was no greater than that adhering to one bead between 3 and 9 hr. Analysis of variance comparing enzymatic activity with respect to time, to number of beads fed, and of time with respect to number of beads fed indicated the differences observed were highly significant (0.005 *P* 0.001, *P* 0.0005, and *P* 0.005, respectively).

Gastric protein secretion (DPM) superficially followed the same pattern as shown for enzymatic activity (Fig. 2A, B). Although initially greater, total secretion in fish with two beads decreased to one-bead levels within 5 hr. Analysis of variance revealed no significant differences between gastric protein secretion with respect to number of beads fed (0.025 *P* 0.01) or with respect to time in terms of number of beads fed (0.1 *P* 0.05). However, trends in gastric protein secretion with respect to time were significant (0.005 *P* 0.001). Papain digestion of gastric juices indicated that 87% of the ^{14}C-amino acid label was recovered in the protein fraction.

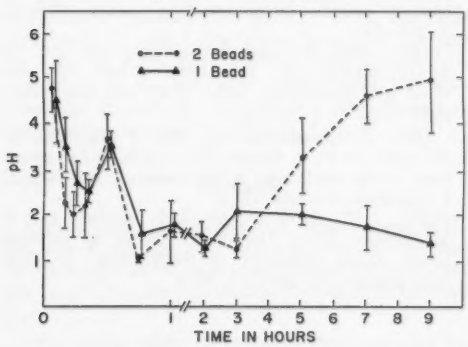

FIG. 1. Acidity (*p*H) of gastric secretions at time of autopsy following ingestion of one (solid line) or two (broken line) glass beads.

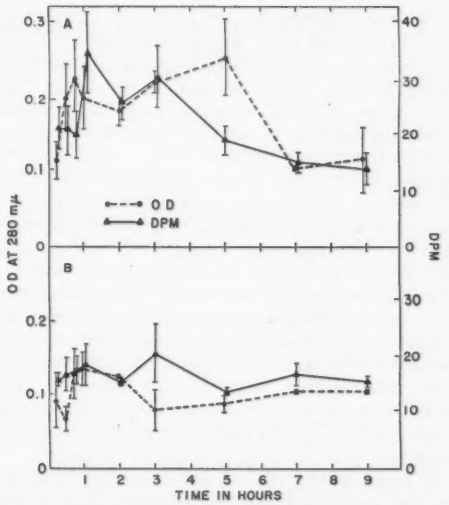

FIG. 2. Pepsin activity expressed as optical density (OD) and ^{14}C-labelled gastric secretion expressed as disintegrations per minute (DPM) per 0.5 ml of hydrolysis products following ingestion of (A) two or (B) one glass bead.

Gastric motility appeared to increase following hand-feeding of the larger volume of material (two beads). Among fish fed one bead and examined 9 hr later, one fish had expelled the bead from the stomach into the intestine. This was remarkable considering the relative diameters of glass bead and pyloric sphincter. Examination of intestines of fish force-fed two glass beads revealed that one fish had passed both beads at 7 hr and two fish had passed both beads at 9 hr.

Discussion

Comparison of these data for bluegill sunfish support the suggestion that, when fish were fed relatively large meals, food material would be forced from the stomach into the intestine prior to completion of gastric digestion (Windell et al. 1969). Windell et al. used pelleted hatchery food having a weight to volume ratio of 62 mg–32.9 mm^3. One glass bead was roughly equivalent in terms of volume to 62 mg of pelleted food and averaged 0.62% (range, 0.33–1.02%) of the fish body weight in experiments where one glass bead was hand-fed. Two glass beads averaged 1.26% (range, 0.76–1.9%) of the fish body weight.

A meal simulated by one bead and representing 0.62% of the fish body weight should be well within the digestive capacities of the bluegill stomach (Windell 1966, 1968; Windell et al. 1969). The pattern of *p*H and enzymatic activity observed following ingestion of one bead probably resembled a normal digestive response (Fig. 1 and 2B). Decreased acidity and relative reduction in enzymatic activity and protein secretion following ingestion of two beads suggested that gastric secretory reserves were exhausted. Reduced enzyme and acid secretion contributed to the marked decrease observed in enzymatic activity, leading to lowered efficiency of gastric digestion. These observations indicate that, under these conditions, incompletely processed food would be passed from the stomach into the intestine where further digestion and assimilation might be impaired. Fecal material produced by these fish might be expected to contain important quantities of undigested nutrients.

It appears that the volume of food entering the fish stomach stimulates secretion of acid and enzyme. There is a maximum secretion rate that can be maintained by the stomach throughout the gastric phase of digestion. A food mass (volume) representing somewhat in excess of 1% of the fish body is too great a volume to be adequately processed and results in exhaustion of the secretory capabilities of the stomach. Incompletely digested food would be passed into the intestine and would appear in the feces. These data suggest that experimentation dealing with efficiencies of converting food to growth of fish should avoid situations where the animals are allowed to feed to satiation (i.e. in excess of 1% of fish body weight per meal). Satiated animals would be doubly handicapped in terms of energy expenditure (depletion of secretory reserves) and reduction in assimilation of ingested nutrients.

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BARRINGTON, E. J. W. 1957. The alimentary canal and digestion, p. 109–161. *In* M. E. Brown [ed.] The physiology of fishes. Academic Press, Inc., New York, N.Y. 447 p.

BERGMEYER, H. U. 1965. Methods of enzymatic analysis. Academic Press, Inc., New York, N.Y. 1063 p.

DAVIS, G. E., AND C. E. WARREN. 1968. Estimation of food consumption rates, p. 204–225. *In* W. E. Ricker [ed.] Methods for assessment of fish production in fresh waters. IBP Handb. 3. Blackwell, Oxford. 313 p.

DOBREFF, M. 1927. Magenverdauung der Haifische: eine Bemerkung uber die Hungerausdauer derselben Fishe. Pfluegers Arch. Gesamte Physiol. Menschen Tiere 217: 221–234. (Cited in Barrington, 1957.)

GLASS, G. B. J. 1968. Proteins in gastric juice, p. 456–477. *In* L. S. Semb and J. Myren [ed.] The physiology of gastric secretion. The Williams and Wilkins Co., Baltimore, Md. 692 p.

PHILLIPS, A. M. 1969. Nutrition, digestion and energy utilization, p. 391–432. *In* W. S. Hoar and D. J. Randall [ed.] Fish physiology, Vol. 1: excretion, ionic regulation, and metabolism. Academic Press, Inc., New York, N.Y. 465 p.

SULLIVAN, M. X. 1907. The physiology of the digestive tract of elasmobranches. Bull. U.S. Bur. Fish. 27: 1–27.

VONK, H. J. 1939. Die biologische Bedetung des *p*H-Optimums der Verdauungs—enzyme bei den Vertebraten. Ergeb. Enzymforsch. 8: 55.

VONK, H. J., AND A. M. W. Mennega, 1938. Das *p*H-Optimum des Pepsins under der *p*H des Mageninhaltes. Acta Brevia Neerl. Physiol. Pharmacol. 8: 27–28. (Cited by Barrington, 1957.)

WINDELL, J. T. 1966. Rates of digestion of the bluegill sunfish. Invest. Indiana Lakes Streams 7: 185–214.

1967. Rates of digestion in fishes, p. 151–173. *In* S. D. Gerking [ed.] The biological basis of freshwater fish production. John Wiley and Sons, Inc., New York, N.Y. 495 p.

1968. Food analysis and rate of digestion, p. 197–203. *In* W. E. Ricker [ed.] Methods for assessment of fish production in fresh waters. IBP Handb. 3. Blackwell, Oxford. 313 p.

WINDELL, J. T., D. O. NORRIS, J. F. KITCHELL, AND J. S. NORRIS. 1969. Digestive response of rainbow trout, *Salmo gairdneri*, to pellet diets. J. Fish. Res. Board Can. 26: 1801–1812.

Lampetra pacifica, a New Nonparasitic Species of Lamprey (Petromyzontidae) from Oregon and California[1]

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VLADYKOV, V. D. 1973. *Lampetra pacifica*, a new nonparasitic species of lamprey (Petromyzontidae) from Oregon and California. J. Fish. Res. Board Can. 30: 205–213.

A new nonparasitic species of Petromyzontidae, *Lampetra pacifica*, from streams of Oregon and California is described and illustrated. This species is distinguishable from *Lampetra richardsoni*, its nearest relative, by fewer myomeres, body proportions, pronounced pigmentation inside the buccal cavity, patterns of pigmentation of the head and tail, and geographical distribution. The description is based on an examination of 233 specimens (43 transformed individuals and 190 ammocoetes).

VLADYKOV, V. D. 1973. *Lampetra pacifica*, a new nonparasitic species of lamprey (Petromyzontidae) from Oregon and California. J. Fish. Res. Board Can. 30: 205–213.

Cet article, accompagné de photographies, décrit une espèce nouvelle de lamproies non parasites, *Lampetra pacifica*, trouvées dans les ruisseaux d'Oregon et de Californie. Cette espèce se distingue de *Lampetra richardsoni*, son congénère le plus rapproché, par les myomères moins nombreux, les proportions du corps, la pigmentation foncée très prononcée de l'infundibulum buccal, la pigmentation restreinte de la tête et de la queue et la répartition géographique. La description est basée sur une étude de 233 spécimens (43 individus transformés et 190 ammocètes).

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SEVERAL authors have referred to occurrence of the European brook lamprey, *Lampetra planeri*, on the western coast of North America, but Vladykov and Follett (1965) showed that in British Columbia, Washington, and in parts of Oregon a distinct species which they named *L. richardsoni* is found, and not *L. planeri*.

Further studies of lampreys from streams of Oregon and California have revealed the existence of another nonparasitic species, never definitely described, for which the name *Lampetra pacifica*, sp. nov., is proposed. "Pacific brook lamprey" is suggested as the common name, after the geographical area of its distribution in the streams of the Pacific coast of the United States.

Methods

Measurements and counts were usually made on the left side of the specimen. Measurements are expressed in percentages of the total lengths of the specimens; the disc length (d) is also expressed in percentage of the branchial length (B_1–B_7). Abbreviations, method of counting trunk myomeres, and definition of stages of maturity of transformed lampreys are as given in Vladykov and Follett (1965), and terminology for types of lamprey teeth is as established in Vladykov and Follett (1967).

In identifying ammocoetes, the number of trunk myomeres, body proportions, and shape of caudal fin were used. Pattern and intensity of pigmentation on head and tail were important in separating species (Vladykov 1950).

Material Examined

The material of *L. pacifica* consists of 43 transformed individuals (41 from Oregon and two from California) and 190 ammocoetes (33 from Oregon and 157 from California).

Data (if known) for each collection appear in the following sequence:

1. Tag number: colors, numbers, and institutions as in Vladykov and Follett (1965).
2. Number of specimens.
3. Sex (in transformed specimen).
4. Total length.
5. Locality.
6. Date of collection.
7. Collector.
8. Repository museum, abbreviated as follows: CAS, California Academy of Sciences, San Francisco

[1]The author prefers the spelling *Petromyzonidae* for the family name.

Printed in Canada (J2611)

NMC, National Museum of Canada, Ottawa
UMMZ, University of Michigan, Museum of Zoology
UW, University of Washington, Seattle.
9. Catalogue number of repository museum.

HOLOTYPE

6864 sW (VDV) (♂, 175 mm): Clackamas River, a tributary of the Columbia River, near Eastacada, Oreg.; April 9, 1964; W. H. Staut; NMC 71–769.

TOPOTYPES

6863, 6865–66 sW (VDV) (1 ♀, 169 mm; 2 ♂, 140–168 mm): collected with the holotype, same date and collector.

PARATYPES

Oregon — (a) Principal collections were made in Crystal Springs Creek, a tributary of the Columbia River, on Reed College Campus, near Portland. Altogether 37 transformed specimens and 33 ammocoetes were obtained:

920–26 B (2 ♀, 105–140 mm; 5 ♂, 116–150 mm), 3600 V, 3671–77 V (8 ammocoetes in transformation, 110–160 mm); spring 1933; L. E. Griffin; UMMZ 132860.

3601–06 V (1 ♀, 99 mm; 5 ♂, 98–132 mm), 3678–79 V (2 ammocoetes, 124–135 mm), 1933 (?).

3880–83 B and 3885 B (5 ammocoetes, 134–148 mm), 3884 B and 3886–97 B (1 ♀, 127 mm; 11 ♂, 115–142 mm); June 13, 1956; Robert W. Phillips.

1510–16 J (4 ♀, 120–163 mm; 3 ♂, 111–138 mm), 1517–18 J (2 ammocoetes, ? mm); April 13, 1957; K. Cockrun.

5210–13 sW (VDV) (2 ♀, 111–124 mm; 2 ♂, 130–135 mm), 5214–15 sW (VDV) (2 ammocoetes, 106–135 mm); spring 1958; K. Cockrun.

1296 J (1 ammocoete in transformation, 119 mm), 1297–98 J (2 ammocoetes, 105–119 mm); October 7, 1963; W. Staut.

4642–43 sW (VDV) (2 ammocoetes, 104–124 mm), without tags (9 ammocoetes, 44–59 mm); February 19, 1966; W. M. Morton and S. G. Jewitt Jr.

(b) 989 B (1 ♂, 98 mm): Willamette River, a tributary of the Columbia River, 3 miles west of Bell Fountain, Benton Co; August 1929; A. D. Welander; UW 3616.[2]

California — (a) 1 female and 135 ammocoetes were collected in Mill Creek, a tributary of the Sacramento River, Tehama County:

2075–R (1 ♀, 129 mm); May 3, 1951; L. H. Carufel.

2214–24 sG (VDV) (16 ammocoetes, 25–116 mm); August 24, 1959; R. J. Hallock.

12766–800 rW (3 ammocoetes in transformation, 114–124 mm; 32 ammocoetes, 72–132 mm) and without tags (13 ammocoetes, 65–122 mm); October 13, 1959; R. J. Hallock.

Without tags (10 ammocoetes, 42–62 mm); October 30, 1959; R. J. Hallock.

648–58 and 660–69 sG (21 ammocoetes, 60–130 mm); November 30, 1959; R. J. Hallock.

445–46 and 499–532 sG (37 ammocoetes, 107–144 mm); January 26, 1960; Department of Fish and Game, Los Molinos, Calif.

570–71 sG (2 ammocoetes, 133–144 mm); April 6, 1960; Department of Fish and Game, Los Molinos, Calif.

Without tags (1 ammocoete, 101 mm); April 27, 1968; S. Pennington; CAS 13568.

(b) 1 female and 6 ammocoetes were collected in Big Chico Creek:

Without tags (1 ♀, 101 mm); April 26, 1950; E. Harris; CAS 13567.

Without tags (5 ammocoetes, 58–91 mm); April 3, 1968; J. D. Hopkirk and party; CAS 13569.

Without tags (1 ammocoete, 75 mm); May 27, 1968; S. Pennington; CAS 13570.

(c) 3817–V (1 ammocoete, 136 mm): Putah Creek, Sacramento River; August 1 ,1954; Collector?

(d) 15 ammocoetes were collected in San Joaquin River, Fresno County:

3834–V (1 ammocoete, 113 mm); May 27, 1940; D. H. Hubbard, UMMZ 132.

589–602 sG (14 ammocoetes, 99–144 mm); August–September 1959; R. Ellers.

Description of *Lampetra Pacifica* sp. nov.

PACIFIC BROOK LAMPREY

Lampetra planeri of authors (western North America, in part); not *Petromyzon planeri* Bloch, 1784, p. 47.

Figures 1–9

DIAGNOSIS

This nonparasitic species of *Lampetra* differs from specimens of *L. richardsoni* in having (1) fewer trunk myomeres, (2) smaller discs, (3) fewer teeth on anterior field, (4) lower dorsal fins, (5) abundance of dark pigmentation in mouth cavity, (6) pronounced dark pigmentation in females over anal finlike fold, and in males along ventral edge of caudal peduncle, and (7) salt and pepper type pigmentation on caudal fin in spawning specimens, instead of solid blotch as in *L. richardsoni*; (8) in ammocoetes, head region and caudal fin less pigmented; and (9) different geographical distribution.

HOLOTYPE

The holotype (tag number 6864 sW-VDV), a male 175 mm in total length when received and subsequently shrunk to 173 mm, is referable to maturity stage 3 (early maturity). Measurements are: prebranchial length, 9.5; branchial length, 9.2; trunk length, 49.1; tail length, 31.8; maximum height of second dorsal fin,

[2]This specimen was identified incorrectly as *L. richardsoni* in an earlier publication (Vladykov and Follett 1965: 143).

FIG. 1. Holotype of *Lampetra pacifica* (Tag sW 6864-VDV), a male in the early stage of maturity, TL 173 mm, Clackamas River, Oreg.

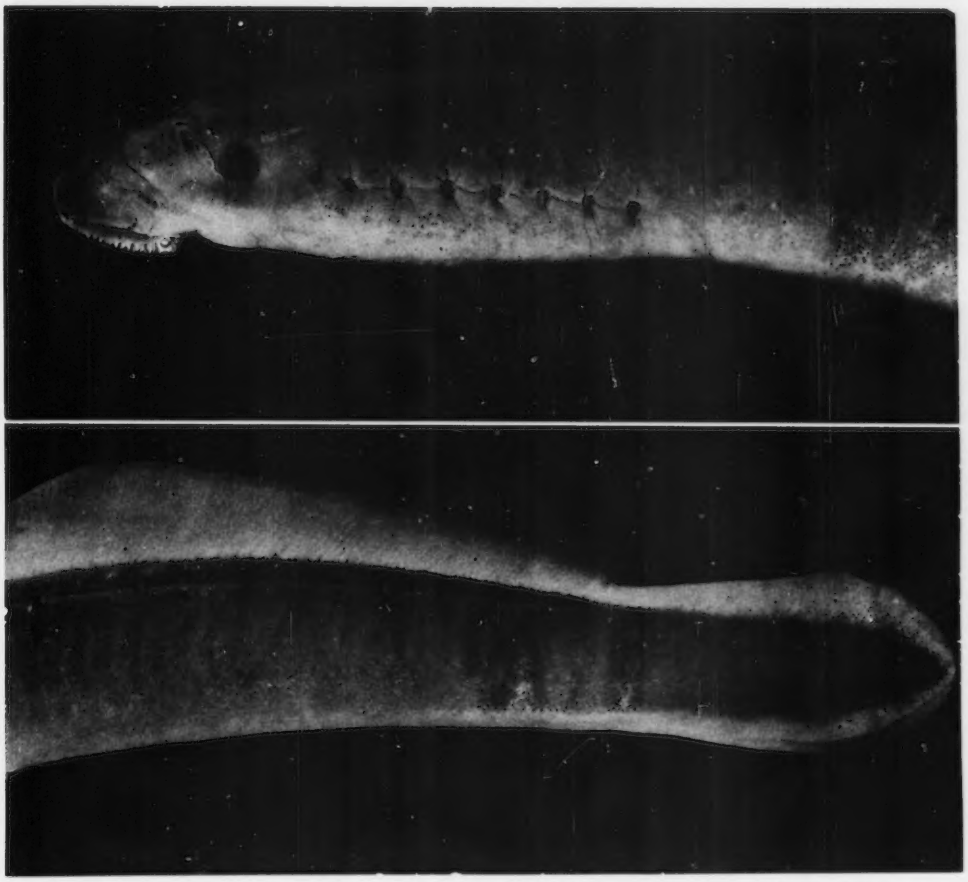

FIG. 2. Enlargements of the head and tail regions of the holotype of *Lampetra pacifica*, TL 173 mm.

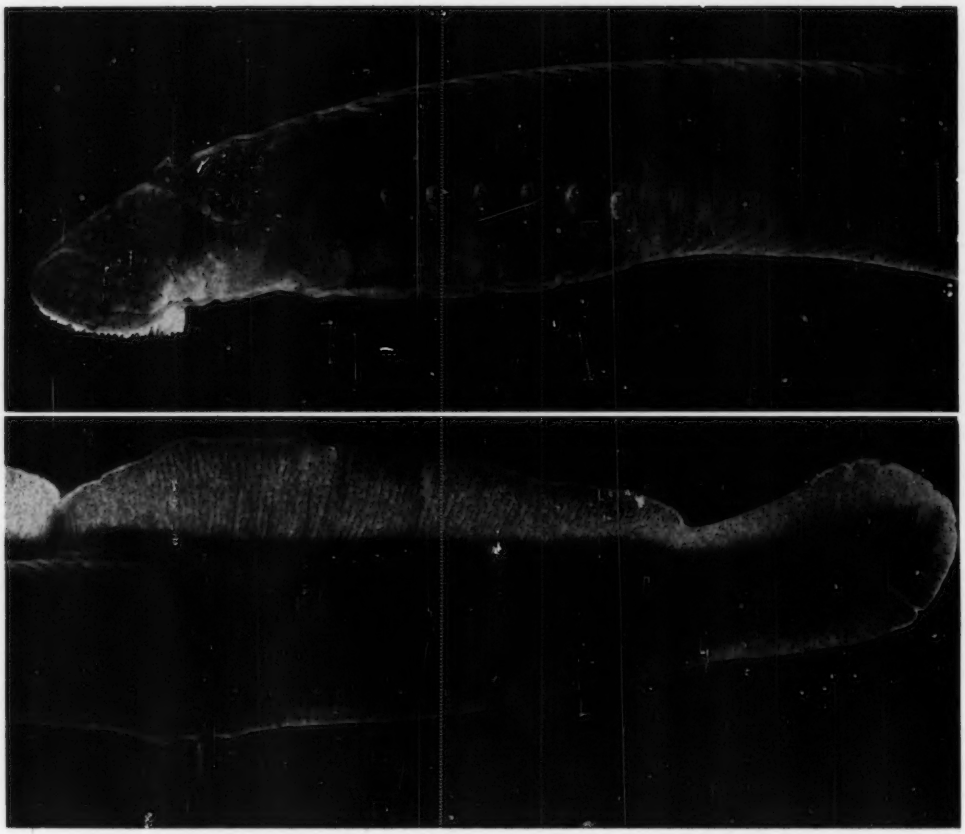

FIG. 3. Enlargements of the head and tail regions of a paratype of *Lampetra pacifica* (Tag sW 5210-VDV), TL 139 mm, male in spawning condition.

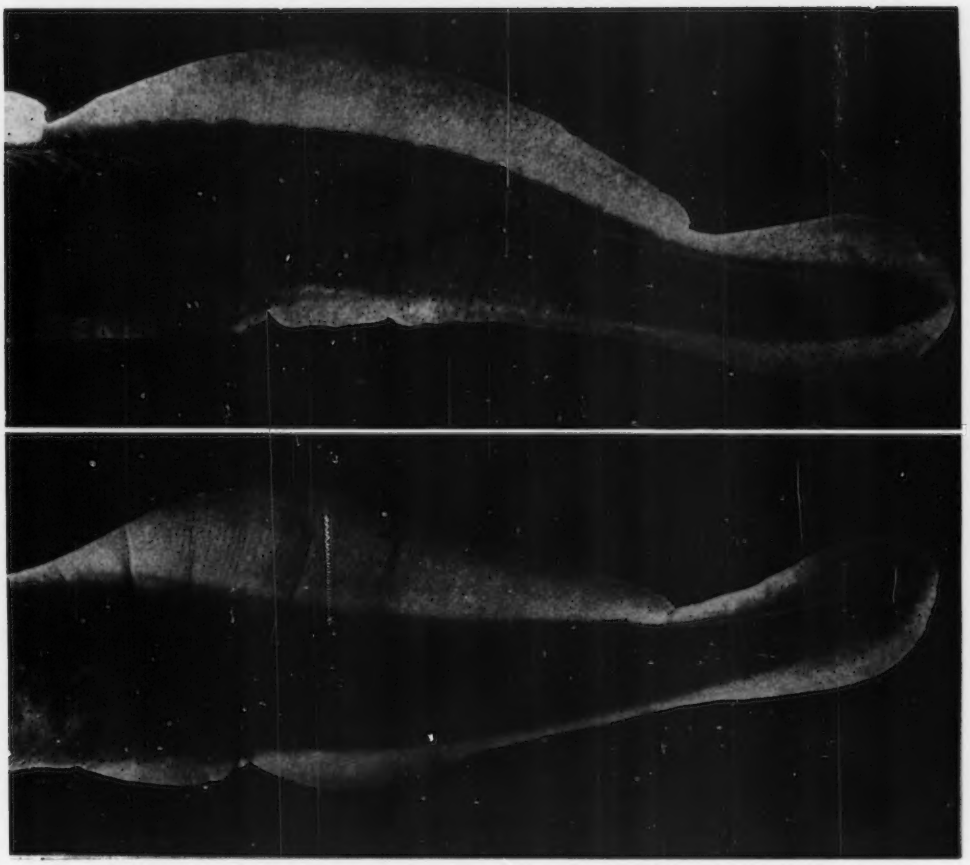

FIG. 4. Enlargements of the tail region of spawning females of *Lampetra*: *upper*) *L. pacifica* (Tag sW 5212-VDV), TL 125 mm; and *lower*) *L. richardsoni* (Tag 4932-R), TL 148 mm.

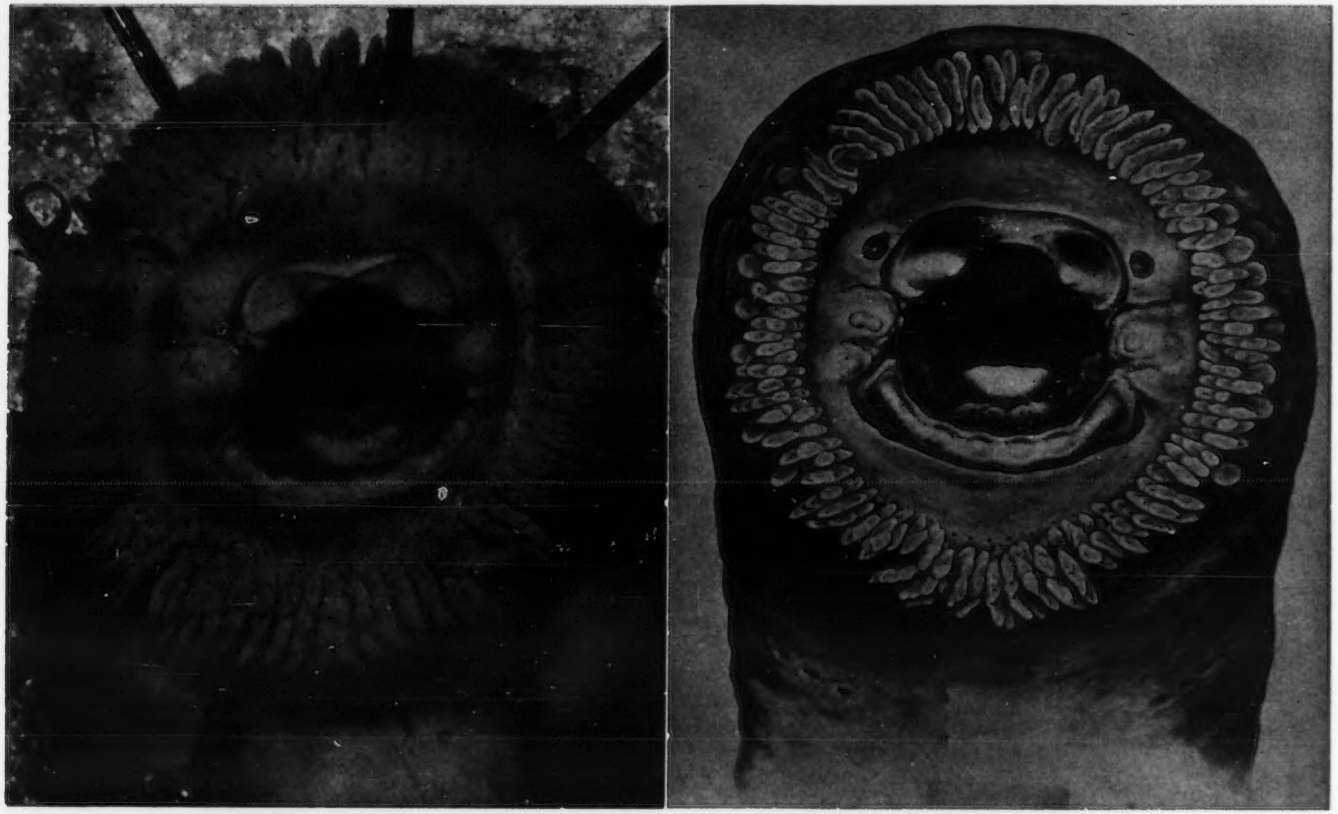

FIG. 5. (*Left*) Enlarged photograph of the disc of the holotype of *Lampetra pacifica*, TL 173 mm, a male in the early stage of maturity; (*right*) A drawing of the enlarged disc of the paratype of *Lampetra pacifica* (Tag 925-B), TL 124 mm, spawning male. Note the obliteration of teeth in the anterior field due to advanced spawning condition.

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FIG. 6. Enlarged photograph of the disc of *Lampetra richardsoni* (Tag 4936-R), prespawning male, TL 134 mm.

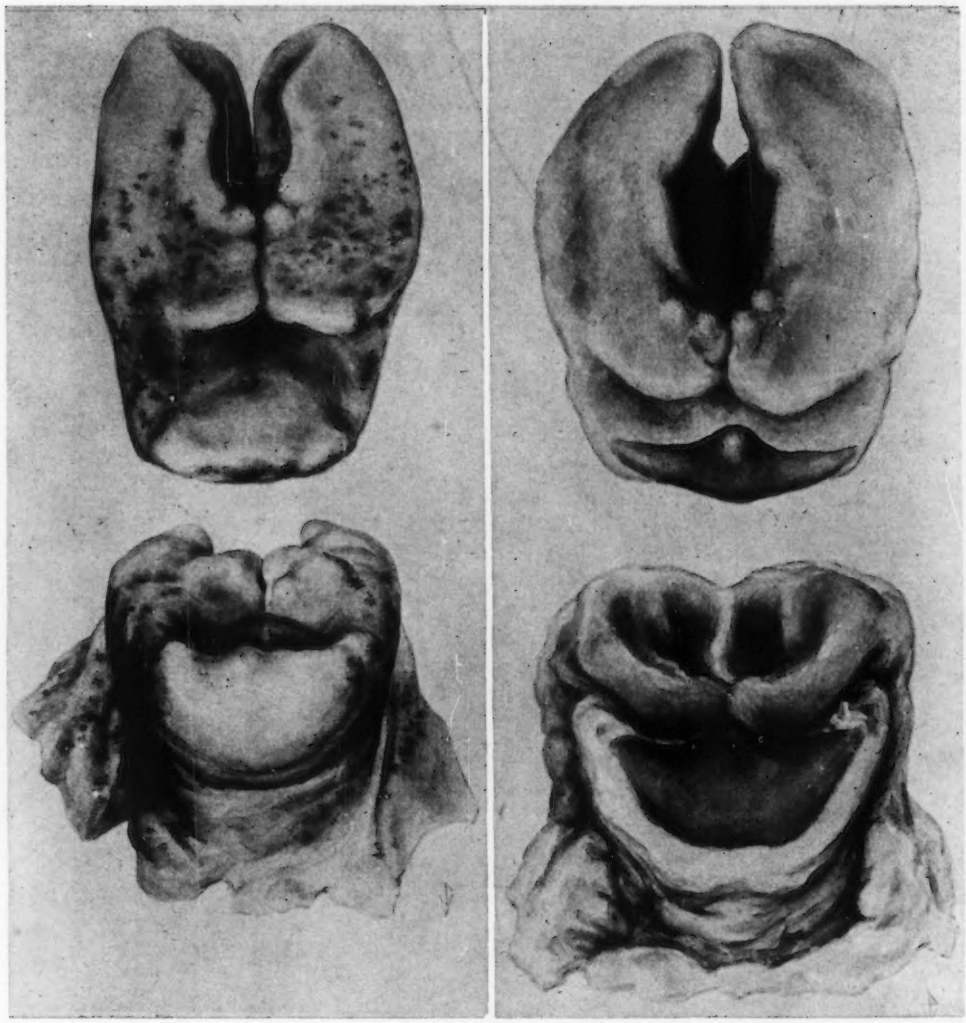

FIG. 7. Enlargements of the tongue with lingual laminae in two species of *Lampetra*: *left*) *L. pacifica* (Tag 1510-J), TL 163 mm, prespawning female with pronounced pigmentation around lingual laminae; *right*) *L. richardsoni* (Tag 4444-R), TL 128 mm, spawning male without pigment.

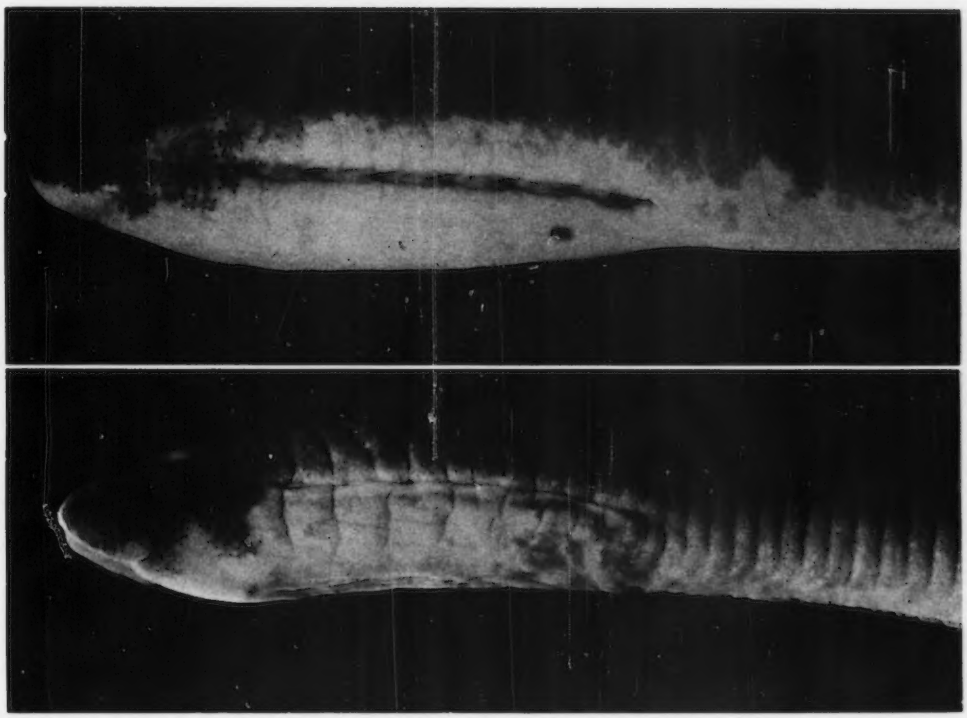

FIG. 8. Enlargements of the head region of ammocoetes of two species of *Lampetra*: *upper*) *L. pacifica* (Tag sG 594), TL 128 mm, from San Joaquin River, Calif.; *lower*) *L. richardsoni* (Tag 3507-V), TL 118 mm, from North Creek, Wash.

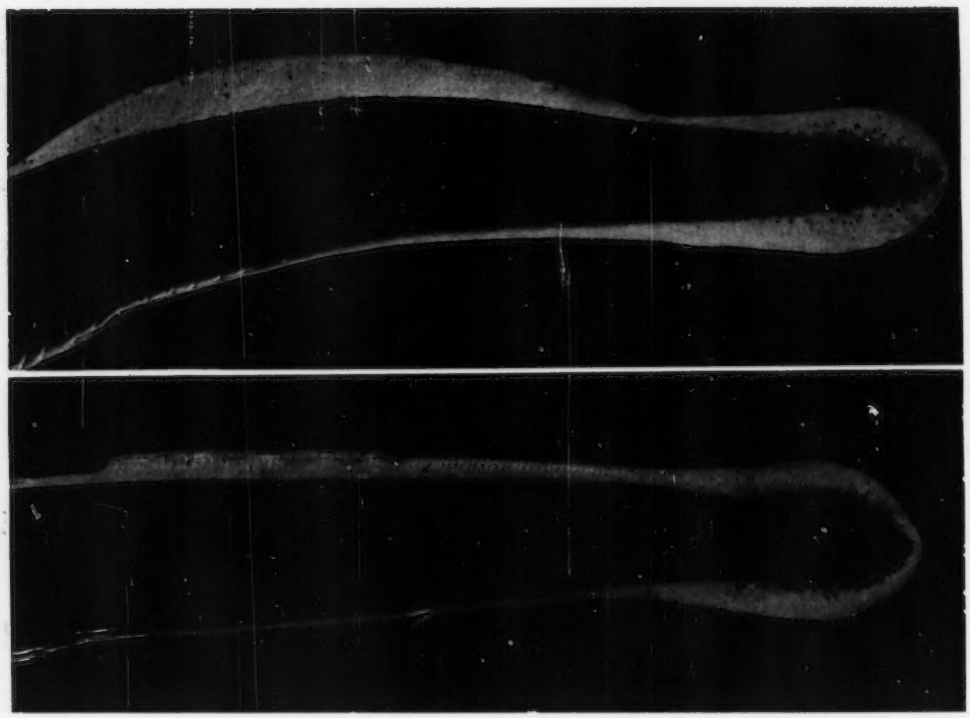

FIG. 9. Enlargements of the tail region of ammocoetes of two species of *Lampetra*: *upper*) *L. pacifica*, the same specimen as that in Fig. 8; *lower*) *L. richardsoni*, the same specimen as that in Fig. 8.

3.2; disc length, 4.6; eye length, 1.4. Disc length in percentage of branchial length is 48.4. There are 55 trunk myomeres.

The teeth were not fully cornified, hence, counts of some of them are uncertain. Inner lateral cusps are three on each side. Anterior and posterior cusps are bicuspid, but middle cusp cannot be defined. On the anterior field, about 10 small teeth; transverse lingual lamina with minute cusps on each side of enlarged median cusp; infraoral lamina with 8 cusps.

Color in 4–5% formalin is dark greyish along the flank and back, and whitish along the ventral surface.

Being in the early stage of maturity, the holotype has two dorsal fins far apart and the genital papilla is barely visible. The pigmentation on the caudal fin is similar to that of an ammocoete (Fig. 3b).

TRANSFORMED SPECIMENS

Total length (*Tables 1–2*) — Lengths of nine prespawning females range from 99 to 169 mm (average 131.4 mm); two spawning females are 101 and 129 mm (average 115 mm). Lengths of six males in early maturity range from 116 to 173 mm (average 148 mm); spawning males were shorter, varying from 98 to 142 mm (average 121.7 mm).

Body proportions (*Tables 1–2*) — Males in early stage of maturity do not have fully developed discs, their diameter or length (as percent TL) being less than that of females (4.8–5.2). Comparing disc length (as percent branchial length), it is 49.5% in immature and 61.9% in mature males, and 51.2% in adult females. During the spawning period, diameter of the male disc is at its maximum. The principal morphological differences between *L. pacifica* and *L. richardsoni* (Tables 1–2) are the greater branchial and tail lengths in the former. *L. pacifica* has a much smaller disc and eye, and the second dorsal fin is lower. The outline of the caudal fin in *pacifica* is rounded, whereas in *richardsoni* it is pointed.

Trunk myomeres — The number of trunk myomeres in transformed *L. pacifica* (Table 3) varies from 53 to 58 (average 55.5), whereas in transformed *L. richardsoni* there are from 60 to 67 (average 63.2).

Dentition — The general pattern of dentition of *L. pacifica* is similar to that of *richardsoni* but the teeth (or cusps) are typically fewer in number and less strongly developed in *pacifica* (Fig. 5). Teeth on the anterior field among specimens of *pacifica* in the early stage of maturity usually number less than 10, while in *richardsoni* they may be more than 25 (Fig. 6). Spawning specimens of *pacifica* completely lose their teeth on the anterior field, while in *richardsoni* the odd tooth remains. The supraoral lamina, which lacks a median cusp, is broad with a blunt cusp on each end, similar to that of *richardsoni*. The number of infraoral cusps in *pacifica* (Table 5)

TABLE 1. Body proportions (in percentages) of prespawning and spawning females of *Lampetra pacifica* from Oregon and California. For comparison, the averages for 16 females of *L. richardsoni* (from Vladykov and Follett 1965) are given below.

Tag no.	Total length (TL) (*mm*)	d–B_1 / TL	B_1–B_7 / TL	B_7–a / TL	a–C / TL	hD_2 / TL	O / TL	d / TL	d / B_1–B_7
				Oregon					
3604V	99	12.7	10.2	53.5	24.3	5.1	3.0	6.0	60.0
926B	105	11.9	10.5	51.0	26.7	4.8	2.4	5.7	54.5
1515J	120	10.8	10.0	50.0	27.5	4.2	2.1	5.8	58.3
3891B	127	10.6	9.4	48.8	27.6	3.9	2.0	5.5	58.3
1514J	131	10.3	9.9	50.8	27.1	3.8	2.3	4.6	46.1
1513J	132	10.6	9.5	50.8	27.3	3.0	2.3	4.5	48.0
922B	140	10.7	10.0	50.0	27.1	2.9	2.1	4.3	42.9
1510J	163	9.8	8.9	50.9	28.5	2.8	1.8	4.6	51.7
6865 sW (VDV)	169	10.1	8.9	48.5	30.8	3.3	1.5	4.1	46.7
Mean	131.4	10.8	9.7	50.5	27.4	3.8	2.2	5.0	51.8
				California					
13567 CAS	101.0	10.4	10.9	52.0	27.2	4.0	1.5	5.0	45.5
2075 R	129.0	10.8	11.2	52.3	25.2	4.6	1.5	5.8	51.7
Mean	115.0	10.6	11.1	52.2	26.2	4.3	1.5	5.4	48.6
Combined mean	123.2	10.7	10.4	51.4	27.2	4.1	1.9	5.2	50.2
L. richardsoni	133.3	11.5	9.6	50.4	26.7	5.1	2.3	5.6	58.1

varies from 6 to 9 (average 8.0), and from 7 to 10 (average 7.7) in *richardsoni*. The cusps on the transverse lingual lamina are smaller than in *richardsoni*, and on the longitudinal lingual laminae are too small to count in both species.

The number of inner lateral cusps in *L. pacifica* in 17 specimens from California and Oregon was as follows: on the right side of the disc — anterior, 1 to 3; middle, 2 to 3; posterior, 1 or 2. On the left side of the disc — usually 1 or 2 in all three positions.

In *pacifica*, as in all *Lampetra* species, the outer laterals and posterials[3] are absent. Of all known nonparasitic species of *Lampetra*, it appears that *L. pacifica* has the least developed dentition on the disc.

Coloration — Our specimens of both *pacifica* and *richardsoni* fixed in 4–5% formalin have the same general coloration. The sides and back are greyish and the lower surface is whitish.

The principal difference between the two species lies in a greater intensity of pigmentation in *pacifica*. In all transformed specimens of *pacifica* there are dark grey chromatophores in the buccal cavity, especially around the lingual laminae (Fig. 11). In *richardsoni*, no such pigmentation is present (Fig. 11), or odd chromatophores may be found deeper in the buccal cavity.

Spawning females of *pacifica* have a pronounced pigmentation over the anal finlike fold, and it is absent in females of *richardsoni* (Fig. 4). In spawning males of *pacifica* there is dark pigmentation along the ventral edge of the caudal peduncle. A striking difference in spawning individuals is found in the pigmentation of the caudal fin: in *pacifica* it is of a "salt & pepper" type (Fig. 3–4), similar to that of *L. fluviatilis* (Vladykov and Follett 1958); in *richardsoni* it is concentrated more or less in a solid mass, making a black blotch of the type described for *L. ayresii* (Vladykov and Follett 1958).

[3]Only Zanandrea (1958) reported the presence of minute posterials in *Lampetra zanandreai*, ("piccoli denti nel labbro inferiore") especially in live specimens. He added two schematic drawings of discs, with a complete set of posterials in one and a partial set in the other. Vladykov (1955) did not notice posterials in any of the 19 transformed specimens. In 1962, Dr Zanandrea told the present author that when he examined freshly caught *L. zanandreai* from the Po River he saw posterials, but when the specimens were put in formalin these teeth disappeared. Based on examination of 1500 adults and 8000 ammocoetes of *L. zanandreai*, Zanandrea (1961) concluded that "all brook lampreys .... of the north Italian plain have the characters on which Vladykov founded the species *L. zanandreai*, i.e. number and disposition of teeth on oral disc, number of trunk myomeres, coloration of ammocoetes and body proportions;" he did not mention the presence of posterials, nor give a drawing of the disc of *L. zanandreai*. Furthermore, he never removed it from the genus *Lampetra*. However, Hubbs and Potter (1971) placed *zanandreai* in the subgenus *Lethenteron*.

Sexual dimorphism — The external appearance of spawning males and females of *pacifica* (Figs. 2–3) is similar to that of other species of *Lampetra*, as described by Vladykov (1955), Vladykov and Follett (1958), and others.

Sexual differences in body proportions of *pacifica* are given in Tables 1–2. Mature males have a greater disc length (d), prebranchial length (d–B_1), and tail length (a–C); the females have a greater trunk length (B_7–a).

Ammocoetes (Table 5)

Total length — Total length of 190 ammocoetes of *L. pacifica* ranged from 54 to 143 mm; the average length of the larger ammocoetes is about 121 mm.

Body proportions — Variations in body proportions of larger ammocoetes from three different localities (Tables 6–8) are summarized in Table 5. Differences in body proportions of ammocoetes of *pacifica* and *richardsoni* of similar average size are: in *pacifica*, prebranchial length (d–B_1) is shorter and tail length (a–C) is longer than in *richardsoni*.

Trunk myomeres — The number of trunk myomeres in 129 ammocoetes of *pacifica* (Table 3) ranges from 52 to 57 (average 54.0). In 121 ammocoetes of *richardsoni*, the number varies from 57 to 65 (average 60.7). Only the counts of 57 and 58 myomeres were common to both species, the 57 count occurring in less than 2% of *pacifica* and in 10% of *richardsoni* ammocoetes.

Pigmentation — Ammocoetes of *pacifica* and *richardsoni* differ in the pattern and extent of dark pigmentation on the tail and certain parts of the head (Table 6).

Caudal fin — Pigmentation of the caudal fin in *pacifica* is restricted to a narrow band around the notochord (Fig. 9). The most common pigmentation, corresponding to weak development (+), was found among 84.4% of ammocoetes; in *richardsoni* the most frequent occurrence was of moderate development type (++) in 55.3% of specimens (Table 6).

Head region — In the head region there are four principal areas of pigmentation of taxonomic significance (Vladykov 1950; 1955).

1. Upper lip. The lower half of the upper lip in ammocoetes of *pacifica* has most frequently only weak pigmentation (+), found in 80.0%; in *richardsoni*, 87.2% have moderate pigmentation (++).

2. Subocular region. Among ammocoetes of *pacifica*, 28.7% have no pigmentation (–) on the subocular region, and 44.5% have only weak pigmentation (+). In contrast, 87.3% of ammocoetes of *richardsoni* have strong pigmentation (+++).

3. Prebranchial region. Among ammocoetes of *pacifica*, 33.3% lack completely the prebranchial blotch and 60.0% have only weak pigmentation (+). In ammocoetes of *richardsoni* 40.4% have moderate pig-

mentation (++) and 51.1% very strong pigmentation (+++).

4. Branchial region. 91.1% of *pacifica* ammocoetes have no pigment along the lower part of the branchial region; in 70.2% of *richardsoni* ammocoetes there is strong pigmentation (+++) in this area (Table 6).

Precursor of tongue — Pigmentation of the precursor of the tongue in ammocoetes of *pacifica* and *richardsoni* (Table 7) does not differ as greatly as pigmentation of the body. In ammocoetes of *pacifica*, absence of pigment (–) or presence of weak pigmentation (+) is of equal frequency, namely 46.7%. Among ammocoetes of *richardsoni*, in 70.6% the pigment is absent (–) and in 23.5% only weakly developed (+).

Geographical Distribution

Lampetra pacifica is found in some freshwater streams of the Pacific drainage of California and Oregon.

TABLE 2. Body proportions (in percentages) of males in early stages of maturity and spawning males of *Lampetra pacifica* from Oregon. Specimen No. 6864 sW (VDV) is the holotype. For comparison, the averages for 29 males of *L. richardsoni* (from Vladykov and Follett 1965) are shown below.

Tag no.	Total length (TL) (*mm*)	d–B_1 / TL	B_1–B_7 / TL	B_7–a / TL	a–C / TL	hD_2 / TL	O / TL	d / TL	d / B_1–B_7
Specimens in early stages of maturity									
924 B	116	12.1	10.3	48.7	26.7	4.3	2.2	5.2	50.0
6866 sW (VDV)	140	10.0	9.7	49.6	30.2	3.2	1.8	4.7	48.1
923 B	142	10.9	9.2	50.4	27.5	3.5	2.1	4.9	53.8
921 B	150	11.0	10.3	50.0	26.7	3.3	2.0	5.0	48.4
6863 sW (VDV)	168	8.9	8.9	46.4	35.0	2.4	1.5	4.2	48.3
6864 sW (VDV)	173	9.5	9.2	49.1	31.8	3.2	1.4	4.6	48.4
Mean	148.0	10.4	9.6	49.0	29.6	3.3	1.8	4.8	49.5
Spawning specimens									
3602 V	98	12.3	11.2	46.9	28.6	4.1	2.6	7.2	63.6
989 B	100	12.0	11.0	46.5	29.0	5.5	2.0	6.0	54.5
3606 V	101	11.9	9.9	48.0	28.7	5.0	2.0	5.9	60.0
3603 V	106	12.3	10.4	47.2	28.3	3.8	2.4	6.6	63.6
3605 V	106	11.8	11.3	45.8	28.3	4.7	2.4	6.6	58.3
1516 J	111	11.7	9.0	59.5	27.0	4.5	2.2	6.3	70.0
3895 B	115	12.2	9.6	47.8	27.8	4.3	2.2	6.1	63.6
3897 B	121	11.2	9.9	46.7	29.8	5.0	2.1	5.8	58.3
3892 B	122	12.7	10.7	45.1	27.1	4.1	2.1	6.6	61.5
3887 B	123	12.2	10.6	46.3	29.3	3.3	2.0	6.5	61.5
3890 B	123	11.8	10.6	44.7	30.1	4.1	2.0	6.5	61.5
925 B	124	12.1	10.5	47.6	29.4	4.0	2.0	6.5	61.5
3888 B	125	12.0	10.4	44.8	30.4	4.0	2.0	6.4	61.5
3884 B	126	12.7	10.3	45.6	30.2	4.8	2.0	6.7	65.4
1512 J	128	12.5	10.5	46.9	28.1	5.5	2.3	6.3	59.3
3893 B	128	11.7	10.5	47.7	28.1	3.9	2.0	6.6	63.0
3889 B	129	11.6	10.1	46.5	29.5	5.0	1.9	6.6	65.4
3601 V	132	12.1	10.2	47.0	29.5	4.9	2.3	6.8	66.7
3894 B	137	12.8	10.9	46.7	27.4	–	2.2	6.9	63.3
1511 J	138	11.6	9.8	47.8	28.3	5.1	2.2	6.5	66.7
920 B	142	11.3	9.9	51.1	26.8	3.9	2.1	6.0	56.7
3886 B	142	11.3	10.6	49.3	27.1	4.2	1.8	6.0	56.7
Mean	121.7	12.0	10.4	47.1	28.6	4.5	2.1	6.5	61.9
Combined mean	127.3	11.6	10.2	47.5	28.8	4.2	2.0	6.1	59.3
L. richardsoni	137.4	12.6	9.8	47.7	28.3	5.6	2.4	6.5	66.7

TABLE 3. Number of trunk myomeres of *Lampetra pacifica* from Oregon and California, and of *L. richardsoni* (from Vladykov and Follett 1965) for comparison. (*t* = transformed; *a* = ammocoetes).

Locality	Date	TL (*mm*)	Stage	No. of specimens	No. of myomeres							Mean
					52	53	54	55	56	57	58	
				Oregon								
Crystal Springs Creek	1933	102–121	*t*	3	–	–	1	–	–	1	1	56.3
”	1933	124–135	*a*	2	–	–	1	1	–	–	–	54.5
”	June 13, 1956	105–142	*t*	6	–	–	–	1	3	2	–	56.2
		133–146	*a*	3	2	–	1	–	–	–	–	52.7
”	Apr. 13, 1957	130–135	*t*	2	–	–	1	1	–	–	–	54.5
”	Spring 1958	111–135	*t*	4	–	–	1	1	1	1	–	55.5
		106	*a*	1	–	–	1	–	–	–	–	54.0
Clackamas River	Apr. 9, 1964	140–173	*t*	4	–	–	–	3	–	1	–	55.5
Total		106–146	*a*	6	2	–	3	1	–	–	–	53.5
		102–173	*t*	19	–	–	3	6	4	5	1	55.7
				California								
San Joaquin River	May 2, 1940	113	*a*	1	–	1	–	–	–	–	–	53.0
San Joaquin River	Aug.–Sept. 1959	99–144	*a*	13	3	4	5	–	1	–	–	53.4
Big Chico Creek	Apr. 26, 1950	101	*t*	1	–	1	–	–	–	–	–	53.0
Big Chico Creek	Apr. 3 & May 27, 1968	75–91	*a*	6	–	1	4	–	1	–	–	54.2
Mill Creek	Aug. 24–Nov. 3, 1959	54–132	*a*	67	2	10	26	28	1	–	–	54.2
”	Jan. 26–Apr. 6, 1960	129	*t*	1	–	–	1	–	–	–	–	54.0
		107–144	*a*	35	4	14	7	8	–	2	–	53.8
”	Apr. 27, 1968	101	*a*	1	–	–	–	–	1	–	–	56.0
Total		54–144	*a*	123	9	30	42	36	4	2	–	54.0
		101–129	*t*	2	–	1	1	–	–	–	–	53.5
Combined total		54–146	*a*	129	11	30	45	37	4	2	–	54.0
		101–173	*t*	21	–	1	4	6	4	5	1	55.5

					57	58	59	60	61	62	63	64	65	66	67	
L. richardsoni		20–175	*a*	121	12	5	15	16	27	23	17	5	1	–	–	60.7
		80–154	*t*	58	–	–	–	4	4	9	16	9	9	5	2	63.2

In California it has been collected in the three tributaries of the Sacramento River: Mill Creek, Big Chico Creek, and Putah Creek. It has also been obtained in the San Joaquin River.

In Oregon it has been found only in the Columbia River basin. It was extensively collected in Crystal Springs Creek near Portland, and some specimens were obtained in the Willamette and Clackamas Rivers.

L. richardsoni has not been found in California. It was reported from Umpqua River near Reedsport, Oregon (Vladykov and Follett 1965). McIntyre

TABLE 4. Number of cusps on infraoral lamina of *Lampetra pacifica*.

Locality	Date	TL (*mm*)	No. of specimens	No. of cusps 6	7	8	9	Mean
Crystal Springs Creek, Oreg.	1933	102–121	3	–	–	2	1	8.3
"	June 13, 1956	105–142	3	1	–	1	1	7.7
"	Apr. 13, 1957	130–135	2	–	1	–	1	8.0
"	1958	111–135	4	–	1	3	–	7.8
Clackamas River, Oreg.	Apr. 9, 1964	140–173	4	–	–	4	–	8.0
Mill Creek, Calif.	May 3, 1951	129	1	–	–	1	–	8.0
Total	1933–1964	102–173	17	1	2	11	3	8.0

TABLE 5. Total length and average body proportions (in percent of TL) of ammocoetes of *Lampetra pacifica* from three localities in California, compared with *L. richardsoni* from Washington (Vladykov and Follett 1965).

Locality	Date	No.	Total length Range (*mm*)	Total length Mean (*mm*)	d-B_1 TL	B_1–B_7 TL	B_7–a TL	a–C TL
Mill Creek	Oct./59	9	89–134	112.5	8.0	12.5	50.8	27.5
Mill Creek	Jan./60	13	101–143	126.0	7.7	12.5	50.8	28.0
San Joaquin River	Aug.–Sept./59	14	98–143	123.9	7.1	11.5	52.6	28.2
Big Chico Creek	Apr.–May/68	5	75–90	83.6	8.8	13.1	49.5	28.0
			Means					
L. pacifica		41	75–143	117.2	7.7	12.2	51.3	28.0
L. richardsoni		53	83–175	113.0	8.5	12.5	51.6	26.2

TABLE 6. Variation in pigmentation on the tail and parts of the head in 45 larger ammocoetes of *L. pacifica* and in 47 of *L. richardsoni*.

	Absent (–) *pacifica*	or Weak (+) *richardsoni*	Moderate (++) *pacifica*	or Strong (+++) *richardsoni*
Tail	41	10	4	37
Upper lip	40	2	5	45
Subocular region	33	1	12	46
Prebranchial blotch	42	4	3	43
Branchial region	45	4	0	43

TABLE 7. Variation in the degree of pigmentation of the precursor of the tongue in large ammocoetes of *Lampetra pacifica* (Oregon and California) and *Lampetra richardsoni* (Washington).

Locality	TL (*mm*)	No. of specimens	Pigmentation of precursor		
			–	+	++
		Lampetra pacifica			
Crystal Spring Creek[a]	132–145	3	2	1	–
Mill Creek, Calif.	81–128	20	9	9	2
San Joaquin River, Calif.	118–134	7	3	4	–
Total	81–145	30	14	14	2
%			46.7	46.7	6.6
		Lampetra richardsoni			
Evans Creek	96–130	5	5	–	–
North Creek	113–120	7	2	4	1
Steel Lake	86–152	5	5	–	–
Total	86–152	17	12	4	1
%			70.6	23.5	5.9

[a]Reed College Campus, Oreg.

(1969) observed a brook lamprey in Berry Creek near Corvallis, Oregon. He called this species *Lampetra planeri*. He kindly sent me one spent and nearly disintegrated female and three ammocoetes (71–88 mm) collected in Berry Creek in May 1968. Pigmentation was difficult to ascertain because of the poor state of preservation. Myomeres observed in the ammocoetes numbered 55, 59, and 59. Identification of these ammocoetes is not certain. I am inclined to consider them as *L. richardsoni*. However, it is not excluded that they may belong to *L. pacifica*. Additional specimens, particularly transformed individuals, are essential for accurate identification.

In my collections there are several ammocoetes of *Lampetra* from streams of California. Some are intermediate in the number of myomeres and pigmentation between *L. pacifica* and *L. richardsoni*.

Two samples are of particular interest:

UMMZ 61, 003; (24 ammocoetes 115–163 mm), Coyote Creek, San José, collected on October 1, 1922, by C. L. Hubbs; and

4409–430–R; (22 ammocoetes 110–177 mm), Garcia River, Mendocino County, collected on August 13, 1952 by J. B. Kimsey.

The ammocoetes from Coyote Creek have from 55 to 59 myomeres (average 57.2). Some of these ammocoetes were studied by Hubbs (1925) who identified them as *L. planeri*: "the number of myomeres varies from 58 to 64, being most frequently 61." However, he counted myomeres "between the last gill opening and the end of the anal slit." Our method of counting, based on Creaser and Hubbs (1922), was between the last gill opening and the anterior tip of the cloacal slit, which probably accounts for the difference.

The ammocoetes from Garcia River with 58–62 myomeres (average 60.3) are close to *L. richardsoni*. However, it is essential to have transformed lampreys for final identification of these and other ammocoetes from California.

The present study permits one to conclude that the areas of distribution of *L. pacifica* are limited to streams of California and Oregon. *L. richardsoni* is principally distributed further north in Washington and British Columbia, and possibly also in Alaska.

It suffices to mention here that *L. pacifica*, together with *L. aepyptera* (Abbott)[4] and *L. zanandreai* Vladykov, belong to a group of *Lampetra* with a small number of myomeres, fewer than 60. The principal difference between these three nonparasitic species, in addition to their geographical distribution, is the degree of dark pigmentation, especially of their ammocoetes. *L. zanandreai* (Vladykov 1955; Zanandrea 1961) is the most pigmented, while *L. pacifica* occupies an intermediate position, and *L. aepyptera* (Vladykov 1950; Seversmith 1953) is least pigmented. Other differences will be discussed in a paper dealing with detailed comparison be-

[4]Hubbs and Potter (1971), on account of "the extreme degeneration and disruption of the teeth," considered this species as belonging to a distinct genus, *Okkelbergia* Creaser and Hubbs.

tween all known species of *Lampetra*, which the present author plans to publish in the near future.

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CREASER, C. W., AND C. L. HUBBS. 1922. A revision of the Holarctic lampreys. Occas. Pap. Mus. Zool. Univ. Mich. 120: 1–14.

HUBBS, C. L. 1925. The life-cycle and growth of lampreys. Pap. Mich. Acad. Sci. Arts Lett. 4: 587–603.

HUBBS, C. L., AND I. C. POTTER. 1971. Distribution, phylogeny and taxonomy, p. 1–65. *In* M. W. Hardisty and I. C. Potter [ed.] The biology of lampreys. Academic Press, Inc., London.

MCINTYRE, J. D. 1969. Spawning behavior of the brook lamprey, *Lampetra planeri*. J. Fish. Res. Board Can. 26: 3252–3254.

SEVERSMITH, H. F. 1953. Distribution, morphology and life history of *Lampetra aepyptera*, a brook lamprey, in Maryland. Copeia 1953: 225–232.

VLADYKOV, V. D. 1950. Larvae of eastern American lampreys (Petromyzonidae). I. Species with two dorsal fins. Natur. Can. 77 (3–4): 73–94.

1955. *Lampetra zanandreai*, a new species of lamprey from northern Italy. Copeia 1955 (3): 215–223.

VLADYKOV, V. D., AND W. I. FOLLETT. 1958. Redescription of *Lampetra ayresii* (Günther) of western North America, a species of lamprey (Petromyzontidae) distinct from *Lampetra fluviatilis* (Linnaeus) of Europe. J. Fish. Res. Board Can. 15: 47–77.

1965. *Lampetra richardsoni*, a new non-parasitic species of lamprey (Petromyzonidae) from western North America. J. Fish. Res. Board Can. 22: 139–158.

1967. The teeth of lampreys (Petromyzonidae): their terminology and use in a key to the Holarctic genera. J. Fish. Res. Board Can. 24: 1067–1075.

ZANANDREA, G. 1958. Posizione sistematica e distribuzione geografica di *Lampetra zanandreai* Vladykov. Mem. Mus. Civ. Stor. Natur. Verona 6: 207–237.

1961. Studies on European lampreys. Evolution 15: 523–534.

The Seasonal Cycle of Vitamin B_{12} in the Strait of Georgia, British Columbia

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Cattell, S. A. 1973. The seasonal cycle of vitamin B_{12} in the Strait of Georgia, British Columbia. J. Fish. Res. Board Can. 30: 215–222.

The seasonal cycle of vitamin B_{12} in the Strait of Georgia, British Columbia, was studied over a 17-month period. The temporal and spatial distribution of this vitamin was found to be more complex than that of nitrate and phosphate in the Strait and B_{12} reported for other areas.

The vitamin data are compared with inorganic nutrient distributions and physical characteristics of the Strait of Georgia to support the hypothesis that the observed heterogeneity of B_{12} is largely the result of vitamin–particulate matter interactions.

Cattell, S. A. 1973. The seasonal cycle of vitamin B_{12} in the Strait of Georgia, British Columbia. J. Fish. Res. Board Can. 30: 215–222.

Le cycle saisonnier de la vitamine B_{12} a été étudié pendant 17 mois dans le détroit de Georgie en Colombie-Britannique. La teneur en vitamine B_{12} varie selon l'endroit et l'époque de l'année de façon plus complexe que les concentrations de phosphate et de nitrate dans le détroit et celle de la vitamine B_{12} dans d'autres régions.

Les concentrations de vitamine ont été comparées à celles des substances nutritives inorganiques en rapport avec les caractéristiques physiques du détroit, afin de vérifier l'hypothèse selon laquelle les variations de concentration de vitamine B_{12} résultent en grande partie d'interactions vitamine–matière solide.

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The significance of vitamin B_{12} in relation to phytoplankton ecology has been debated for a number of years (Daisley 1957; Droop 1957a, b, 1968; Provasoli 1963; Provasoli and Pintner 1953). While this has prompted widespread investigation of the vitamin B_{12} requirements of a number of phytoplankton species (e.g. Lewin 1954; Sweeney 1954; Droop 1955, 1968; Droop et al. 1959; Gold 1962; Ryther and Guillard 1962; Guillard and Cassie 1963; Guillard 1968; Carlucci and Silbernagel 1969; Carlucci and Bowes 1971b), our knowledge of the seasonal cycle of B_{12} in the marine environment is very limited. The studies of Cowey (1956) in the North Sea, Vishniac and Riley (1961) in Long Island Sound, and Menzel and Spaeth (1962) in the Sargasso Sea represent the sum total of annual cycle data on vitamin B_{12} in the sea.

In the present study the dissolved vitamin B_{12} cycle in the Strait of Georgia was studied over a 17-month period and found to be more complex than those of the studies cited above. The results are compared with nutrient and physical data from the Strait of Georgia in an attempt to identify factors affecting this distribution.

Materials and Methods

Samples were collected from four stations in the Strait of Georgia (1–4) and one station (5) in the connecting waters between the Strait of Georgia and Juan de Fuca Strait during 17 cruises between February 1967 and September 1968. The station positions were: Station 1, 50°00′N125°05′W; Station 2, 49°40′N124°35′W; Station 3, 49°15′N123°30′W; Station 4, 48°50′N123°03′W; and Station 5, 48°23′N123°20′W. The four stations in the Strait of Georgia were selected to typify the range of physical properties, temperature, salinity, and mixing in the surface waters (Tully and Dodimead 1957; Waldichuk 1957). Station 5 was chosen to monitor changes in nutrient conditions in waters flowing between the two straits. It was not possible to sample at all stations during May, June, and July 1967 and February 1968 due to ship operation and scheduling difficulties.

Samples were collected with plastic National Institute of Oceanography (N.I.O.) bottles from six depths (surface, 5, 10, 20, 50, and 100 m) at Stations 1–4 and five

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depths (surface, 5, 10, 20, and 50 m) at Station 5. Three hundred milliliters of each sample were filtered through an 0.22μ GS Millipore® filter and frozen in sulphuric–chromic acid cleaned polyethylene containers for nutrient analyses. Salinity and temperature measurements were also made on each sample.

Soluble vitamin B_{12} concentration was determined by bioassay with *Amphidinium carterae*, using a modified version of Antia's method (Antia et al. 1963). All glassware used in the bioassay was cleaned in chromic–sulphuric acid solution, rinsed with distilled water, and autoclaved for 15 min at 15 psi. Culture medium, vitamin B_{12} solutions, samples, and vitamin-free sea water were sterile-filtered through 0.22μ GS Millipore® filters. Vitamin-free sea water was prepared according to the activated charcoal method of Ryther and Guillard (1962).

Sterile samples were dispensed in 10-ml aliquots into 50-ml Pyrex screwcap flasks that had been baked on a hot plate for 2 hr at 400 C. Each sample was diluted with 10 ml of vitamin-free sea water (31‰) to minimize salinity extremes. External standards containing 1, 2, 3, 5, and 10 ng/liter of vitamin B_{12} were run with each bioassay series. Samples, standards, and vitamin-free seawater blanks were set up in triplicate and innoculated with 1 ml each of sterile enrichment medium and B_{12} depleted *Amphidinium* culture.

The bioassay cultures were incubated below a bank of "daylight" fluorescent bulbs with a rating of 0.15 ly/min. Overhead illumination was found to be the most satisfactory arrangement, as *A. carterae* is positively phototactic and tends to stick to the flask wall with either side or bottom illumination. The cultures were maintained at ambient room temperature of about 24 C.

Vitamin B_{12} concentrations were calculated on the basis of final yield determinations. The cultures reach this state after 11 or 12 days of incubation. Ten milliliters of the bioassay sample were diluted to 200 ml with filtered sea water and then counted on a Model B Coulter Counter to determine the final yield. Five 20-sec counts were made on each sample. Replicate counts were always within $\pm 5\%$ of the mean. The final yield of cell numbers of *A. carterae* was linearly related to vitamin B_{12} concentrations of at least 10 ng/liter as determined by the standards. A least squares regression line, calculated from the mean standard values of each bioassay series, was used to estimate vitamin B_{12} concentration of the samples.

Reactive phosphorus and nitrate–nitrite nitrogen concentrations were determined in the surface, 5-, and 10-m water samples using the methods described in Strickland and Parsons (1965). A modified cadmium reduction column described by Wood et al. (1967) was employed for the nitrate measurements.

Results

The temporal distribution of temperature and salinity in the upper 100 m is shown in Fig. 1 for Station 3. This station exemplified the maximum range in values for these parameters because of its proximity to the Fraser River. Variation in both salinity and temperature decreased with distance from the Fraser River, being minimal at Station 5 in the Juan de Fuca Strait.

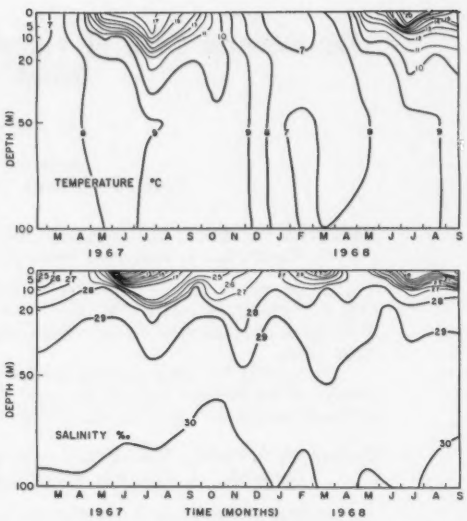

FIG. 1. Temporal cycle of temperature (C) and salinity (‰) at Station 3 in the Strait of Georgia.

The water column in the Strait of Georgia becomes highly stratified in the upper 20 m during the summer months not only because of increased insolation but also largely because maximum freshwater runoff (particularly from the Fraser River) occurs during June or July (Tully and Dodimead 1957; Waldichuk 1957). While this salinity effect is maximal at Station 3, lowered salinities were measured in the surface waters of all four stations in the Strait of Georgia during this time period.

The nitrate and phosphate data are presented in Table 1 and the average values in Fig. 2. High concentrations of both nutrient species were noted from October through February at all stations. The values for both nutrients remained high at Station 5 all year, but showed a marked decrease in the Strait of Georgia during the spring months. Some recovery was noted in April 1968, but by May of both years concentration of both nutrients were low at all the Strait of Georgia stations and showed extreme stratification between the surface and 10-m depth with the development of the pycnocline. These conditions persisted through September until the breakdown of the pycnocline, at which time the nutrient concentrations began to increase towards the winter high. The correlation of nitrate and

phosphate concentration with water density (sigma-*t*) is shown in Table 2.

The monthly averages for vitamin B_{12} concentration in the upper 10 m of the Strait of Georgia (Fig. 2) show some similarity in temporal distribution to that of the inorganic nutrients, including a winter high in concentration followed by an early spring decrease with a slight peak in concentration during the late spring. A distinct difference, however, is apparent during the summer with B_{12} showing a peak both years while the inorganic nutrients were at minimal concentrations. Furthermore, the average values of B_{12} during the spring and summer of 1968 were about twice as high as the 1967 values.

Comparison of B_{12} and inorganic nutrient concentrations at individual sampling depths (Table 1 and 3) reveal further differences between vitamin and inorganic nutrient distributions in the Strait of Georgia. While the inorganic nutrient concentrations showed a marked tendency to increase with depth and were significantly correlated with sigma-*t* throughout much of the year, B_{12} showed little significant correlation with this parameter particularly during the summer months when stratification was so strongly developed (Table 2). Thus, there was no clear-cut picture of vitamin concentration increasing (or decreasing) with depth as occurred with the inorganic nutrients.

Discussion

The range in B_{12} concentration recorded in this study is similar to that found in other coastal regions (e.g. Droop 1954; Lewin 1954; Cowey 1956; Vishniac and Riley 1961; Carlucci 1970, 1971; Natarajan 1970; Carlucci and Bowes 1971a). The temporal and spatial pattern of distribution in this study, however, was more complex than has previously been recorded. Cowey (1956) found only one yearly peak in B_{12} concentration, which occurred during the winter months in the North Sea. Menzel and Spaeth (1962) also noted only one annual maximum in the Sargasso Sea. Vishniac and Riley (1961) reported two concentration maxima in Long Island Sound; one in the late summer and another during the winter months, which corresponded reasonably well with the inorganic nutrient pattern.

The winter peak in vitaman B_{12} concentration in the Strait of Georgia corresponds to that found in temperate region studies cited above and with the yearly high in inorganic nutrient concentration in the Strait and appears to be related to normal regenerative processes associated with the breakdown of the seasonal pycnocline. The small spring increase of both B_{12} and inorganic nutrients in the Strait followed the spring plankton bloom (Parsons et al. 1969) and was likely due to nutrient regeneration of this biological material. That B_{12} concentration began to increase before either nitrate or phosphate may be explained either as the result of direct algal production of B_{12} (Carlucci and Bowes 1970a, b) or more rapid regeneration of the vitamin or both.

The uniqueness of the B_{12} pattern in the Strait of Georgia is associated with the summer maximum, which deviated strongly from the inorganic nutrient pattern for this time period during both years of the study. It does not seem possible that these peaks could have been generated by regeneration of biological material because there was no concomitant production of inorganic nutrients. The water column was highly stratified during this time period precluding any water-column turnover, which in any event would have also resulted in inorganic nutrient increase. Another possibility would be some external input into the Strait.

External sources of B_{12} could include influx of the vitamin from either end of the Strait or Fraser River runoff or both. Advection of sea water into the Strait apparently occurs throughout the year (Tully and Dodimead 1957; Waldichuk 1957; Dodimead and Pickard 1967) but, because of its greater density, this water tends to sink to the lower zones of the water column. Furthermore, there was no evidence of particularly high B_{12} concentrations at either Station 1 or 5 during this time period, nor any significant correlation with water density (Table 3), suggesting that this was not a likely source of the vitamin.

Fraser River discharge into the Strait of Georgia reaches an annual maximum during June or July (Tully and Dodimead 1957; Waldichuk 1957), which corresponds approximately to the summer B_{12} peak. This summer discharge is very turbid due to enhanced silt load and can be seen fanning out over much of the central portion of the Strait. For example, in June 1967, discolored runoff water was noted nearly as far north as Station 2 and as far south as Station 4. Previous studies have shown that the B_{12} content of suspended particles in turbid coastal and river waters can be quite high (Burkholder and Burkholder 1956, 1958; Starr 1956), containing up to 6400 ng B_{12}/g of solid material that can be released into the water column by microbial action. This appears to be the only appreciable exogenous source of B_{12} in the Strait of Georgia during the summer and the fact that B_{12} can be released from silt particles would account for not only the summer peak in concentration but also the lack of correlation between vitamin concentration and sigma-*t* as well.

The possible role of Fraser River runoff in supplying B_{12} to the Strait of Georgia may be demon-

TABLE 1. Inorganic nitrate–nitrite (μg-at N/liter) and phosphate (μg-at P/liter

Station	Depth (*m*)	1967 Feb. 20–22 P	Apr. 25–27 P	May 22–23 P	June 12–13 P	July 26–28 N	July 26–28 P	Sept. 25–27 N	Sept. 25–27 P	Oct. 25–26 N	Oct. 25–26 P	Nov. 27–28 N	Nov. 27–28 P	Jan. 9–11 N	Jan. 9–11 P
1	0	1.6	1.5	0.1	0.1	0.6	0	5.9	0.9	6.2	1.8	20.3	2.1	15.4	2.2
	5	2.2	1.6	0.3	0.4	0.4	0	6.2	1.5	5.3	2.0	19.0	2.1	16.0	2.2
	10	2.1	–	–	1.5	8.9	1.2	12.2	1.8	6.4	2.1	20.2	2.1	16.5	2.1
	$\bar{X}$	2.0	1.6	0.2	0.7	3.3	0.4	8.1	1.4	6.0	2.0	19.8	2.1	16.0	2.2
2	0	3.1	1.6	0.1	0.2	0.7	0.5	2.4	1.0	11.5	1.9	18.1	1.9	15.4	2.2
	5	2.9	1.7	0.9	0.3	0.4	–	4.5	1.8	11.8	1.7	17.9	1.9	15.6	2.3
	10	4.0	–	–	1.5	0.4	0.2	7.4	1.4	13.0	1.1	17.3	1.9	16.3	2.3
	$\bar{X}$	3.3	1.7	0.5	0.7	0.5	0.4	4.8	1.4	12.1	1.6	17.8	1.9	15.8	2.3
3	0	2.8	0.9	0.4	0.9	–	0.1	2.5	1.6	13.0	1.6	15.3	1.7	16.8	2.4
	5	2.6	1.4	0.4	1.6	–	0.1	7.8	1.3	13.4	0.8	15.6	1.7	16.0	2.2
	10	2.2	–	–	1.9	–	0.3	10.9	1.8	14.3	1.1	16.0	1.8	15.9	2.2
	$\bar{X}$	2.5	1.2	0.4	1.5	–	0.2	7.1	1.6	13.6	1.2	15.6	1.7	16.2	2.3
4	0	2.1	0.7	–	0.2	–	0.6	4.6	1.8	14.5	1.1	13.9	1.7	15.9	2.2
	5	2.3	1.4	–	0.3	–	1.1	7.3	1.4	16.6	1.9	14.1	1.7	16.3	2.2
	10	1.7	–	–	1.4	–	1.6	7.9	2.8	19.2	2.0	14.6	1.8	16.0	2.2
	$\bar{X}$	2.0	1.1	–	0.6	–	1.1	6.6	2.0	16.8	1.7	14.2	1.7	16.1	2.2
5	0	1.6	1.9	–	–	–	1.8	11.0	2.9	18.6	2.4	14.5	2.0	20.1	2.1
	5	2.0	1.4	–	–	–	1.8	11.1	2.1	22.0	2.4	16.7	2.1	20.7	2.1
	10	1.5	–	–	–	–	–	11.9	2.1	21.2	2.3	17.9	2.0	21.0	2.0
	$\bar{X}$	1.7	1.7	–	–	–	1.8	11.3	2.4	20.6	2.4	16.4	2.0	20.6	2.1

strated by the results obtained at Station 3 in the summer of 1967. In June 1967, the surface salinity at Station 3 was very low (3.8‰) due to the intrusion of runoff water, but increased to 26.9‰ at 10 m. The surface was a muddy brown color due to the presence of silt particles. Silt also discolored the 5-m sample, while at 10 m and below the water was virtually free of silt as determined by microscopical examination. The extreme salinity stratification in the upper 10 m and the lack of silt below 5 m sug-

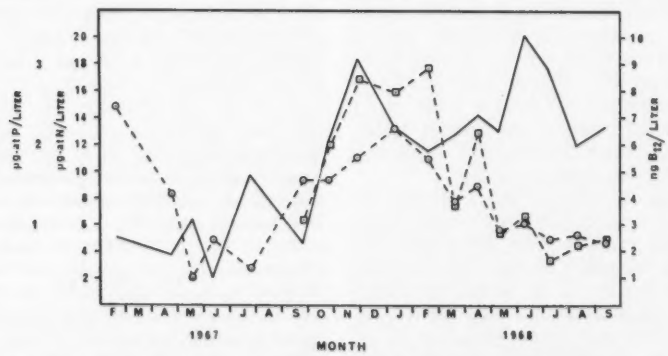

FIG. 2. Average values of nitrate–nitrite (□-----□), phosphate (○-----○), and vitamin B_{12} (——) in the upper 10 m of the Strait of Georgia.

oncentration in the Strait of Georgia (Stations 1–4) and Juan de Fuca Strait (Station 5).

1968																	
Feb. 20–21		Mar. 19–21		Apr. 16–18		May 10–12		June 7–8		July 8–9		Aug. 10–11		Sept. 13–14			
N	P	N	P	N	P	N	P	N	P	N	P	N	P	N	P	N	P
–	–	1.2	0.7	12.6	1.8	5.1	0.7	3.3	0.9	0.4	0.2	2.1	0.5	2.9	0.4	6.3	1.0
–	–	4.6	1.0	12.0	1.9	9.0	1.2	6.3	1.1	1.7	0.3	0.8	1.3	1.0	0.3	6.9	1.2
–	–	6.7	1.5	12.4	1.8	10.7	1.5	14.2	1.8	14.3	1.0	11.8	1.6	1.5	0.5	11.3	1.6
–	–	4.2	1.1	12.3	1.8	8.3	1.1	7.9	1.3	5.5	0.5	4.9	1.1	1.8	0.4	8.2	1.3
17.6	2.0	7.2	1.3	13.3	1.5	0.9	0.3	3.3	0.8	0.6	0.1	0.8	0.6	0.7	0.4	7.1	1.2
17.8	2.0	6.0	1.5	14.9	1.4	7.9	9.0	4.3	0.8	7.4	0.3	0.4	0.4	0.8	0.5	8.4	1.2
18.2	1.9	6.0	1.6	14.5	1.4	13.2	1.5	10.7	1.5	6.8	0.6	0.5	0.5	4.0	0.7	9.9	1.5
17.9	2.0	6.4	1.5	14.2	1.4	10.1	0.9	6.1	1.0	4.9	0.3	0.6	0.5	1.8	0.5	8.6	1.3
16.1	1.6	1.9	0.5	14.0	1.3	0.9	0.6	0.8	0.3	1.7	0.2	0.4	0.2	0.7	0.4	7.0	1.0
17.4	1.7	5.9	1.2	14.7	1.5	0.3	0.7	6.6	1.2	1.3	0.2	0.5	0.5	0.4	0.3	8.3	1.1
19.8	1.9	10.0	2.1	14.0	1.5	6.1	1.4	10.9	1.1	1.2	0.6	0.4	0.7	6.7	1.1	10.5	1.4
17.8	1.7	5.9	1.3	14.2	1.4	2.4	0.9	6.1	0.9	1.4	0.3	0.4	0.5	2.6	0.6	8.6	1.2
–	–	12.2	1.4	10.5	1.3	1.2	0.5	0.5	0.2	0.3	0.2	7.8	1.2	10.8	1.5	8.4	1.1
–	–	12.1	1.5	12.9	1.5	2.8	0.8	5.4	1.1	1.6	0.9	14.4	1.7	13.6	1.6	10.6	1.4
–	–	15.0	1.7	9.4	1.2	6.7	1.3	11.4	1.5	10.3	1.3	14.7	1.7	15.8	1.8	12.8	1.7
–	–	13.1	1.5	10.9	1.3	3.6	0.9	5.8	0.9	4.1	0.8	12.3	1.5	13.4	1.6	10.6	1.4
–	–	14.5	1.7	9.9	1.1	16.0	2.2	16.4	1.7	15.2	1.7	20.0	2.3	16.9	1.9	15.7	2.0
–	–	16.3	2.0	8.8	1.0	18.0	2.5	15.9	1.6	15.8	1.8	20.1	2.3	17.7	2.0	16.6	1.9
–	–	16.1	1.7	17.5	1.6	16.4	2.2	18.5	2.0	16.0	1.7	20.5	2.6	17.7	1.9	17.7	2.0
–	–	15.6	1.8	12.1	1.2	16.8	2.3	16.9	1.8	15.7	1.7	20.2	2.4	17.4	1.9	16.7	1.9

TABLE 2. Correlation coefficients for vitamin B_{12}, phosphate, and nitrate with water density gradients (sigma-*t*) in the upper 10 m of the Strait of Georgia. (*Significant at 5% probability level, **Significant at 1% probability level.)

Date	B_{12}	PO_4	NO_3
	1967		
Sept. 25–27	.51	.52	.94**
Oct. 25–26	.71**	.78**	.42
Nov. 27–28	–.52	.80**	.30
	1968		
Jan. 9–11	.11	.66**	.79**
Feb. 20–21	.06	.96**	.64*
Mar. 19–21	.38	.75**	.69**
Apr. 16–18	–.85**	–.36	–.28
May 10–12	–.46	.81**	.84**
June 7–8	–.09	.89**	.85**
July 8–9	–.40	.80**	.82**
Aug. 10–11	.20	.87**	.78**
Sept. 13–14	–.30	.73**	.73**

gests that the runoff water had only recently penetrated the water column at Station 3. B_{12} concentrations were low during June at Station 3, ranging from 0.1 ng/liter at the surface to 1.7 ng/liter at 10 m and undetectable at 100 m.

Runoff water was still apparent at Station 3 in July 1967. However, the surface salinity had increased to 12.8‰ at the surface, indicating that some mixing with Strait of Georgia water was taking place. Silt was still abundant in the upper 5 m of the water column but not so abundant as to discolor the water. Lesser amounts of silt particles were now evident in the other samples to a depth of 50 m. The concentration of B_{12} at the surface remained low (0.8 ng/liter) but increased to 12.5 ng/liter at 5 m near the pycnocline. B_{12} concentration was also above 10 ng/liter at 20- and 50-m depth. These were the highest values recorded in the Strait during the summer months.

An apparent inconsistency with this interpretation is that B_{12} was always present in very low concentrations in runoff water with high silt content. These low concentrations cannot be attributed to a salinity effect on the growth of the bioassay orga-

TABLE 3. Vitamin B_{12} concentration (ng/liter) in the Strait of Georgia (Station 1–4) and Juan de Fuca Strait (Station 5) from February, 1967, to September, 1968.

		1967								1968									
Station	Depth (*m*)	Feb. 20–22	Apr. 25–27	May 22–23	June 12–13	July 26–28	Sept. 25–27	Oct. 25–26	Nov. 27–28	Jan. 9–11	Feb. 20–21	Mar. 19–21	Apr. 16–18	May 10–12	June 7–8	July 8–9	Aug. 10–11	Sept. 13–14	$\bar{X}$
1	0	2.5	1.1	3.5	2.7	1.5	1.6	5.2	11.0	6.7	–	2.3	5.9	3.7	4.9	2.6	3.1	5.4	4.0
	5	3.5	1.6	2.3	0.8	5.5	1.9	8.1	9.2	6.8	–	3.5	8.7	5.1	5.2	7.1	6.3	6.4	5.1
	10	4.0	1.4	4.4	1.5	6.1	3.6	9.3	10.8	5.6	–	5.1	8.4	5.8	6.5	7.9	6.6	6.7	5.9
	20	2.1	3.4	4.1	0.7	6.0	3.1	10.0	8.9	3.9	–	4.8	8.5	8.0	5.5	9.8	7.4	7.0	5.8
	50	0.8	3.9	3.1	1.1	7.3	3.2	9.1	10.4	5.1	–	8.4	7.6	8.6	4.2	8.8	4.9	5.5	5.8
	100	–	4.2	4.2	1.1	5.3	3.7	8.7	8.4	4.8	–	6.8	11.5	8.8	4.5	9.7	5.6	6.8	6.3
	$\bar{X}$	2.6	2.6	3.6	1.3	5.3	2.9	8.4	9.8	5.5		5.2	8.4	6.7	5.1	7.7	5.7	6.3	
2	0	3.5	2.8	2.5	2.9	2.1	1.3	6.4	13.1	10.0	5.9	5.6	6.9	6.4	7.7	9.3	4.5	6.7	5.7
	5	3.1	2.9	3.4	1.4	3.1	1.3	5.2	14.6	6.6	5.4	7.5	6.2	5.4	8.0	12.0	5.3	6.9	5.8
	10	3.6	1.9	4.0	0.4	3.0	2.8	7.7	11.7	5.7	5.9	11.5	7.6	9.3	17.1	12.5	5.5	7.7	6.9
	20	3.8	3.1	4.3	0.1	6.3	2.2	5.8	10.7	5.8	4.4	5.9	8.0	9.3	8.7	11.0	7.0	8.8	6.2
	50	0	3.0	4.6	0	9.6	3.7	10.6	10.4	4.9	8.8	8.7	9.2	10.3	8.9	12.6	9.7	10.3	7.4
	100	0	4.3	4.7	0.6	5.6	2.3	9.6	8.7	5.4	3.8	4.7	8.6	10.4	8.2	10.3	8.6	8.5	6.1
	$\bar{X}$	2.3	3.0	3.9	0.9	5.0	2.3	7.6	11.5	6.4	5.7	7.3	7.8	8.5	9.8	11.3	6.8	8.2	
3	0	3.2	2.1	2.1	0.1	0.7	1.3	2.8	10.1	7.7	6.1	4.9	9.4	6.6	9.3	7.0	6.2	6.5	5.1
	5	3.0	1.5	1.9	1.1	12.5	1.6	3.8	9.0	7.6	4.6	3.0	10.5	5.9	18.7	16.4	7.3	6.3	6.8
	10	1.3	2.0	4.7	1.7	6.0	2.2	5.9	11.0	6.2	6.6	8.7	7.5	6.2	9.7	14.3	6.3	8.3	6.4
	20	1.0	1.9	5.0	0.1	10.2	4.3	12.0	9.7	5.5	4.7	6.3	11.1	6.5	8.5	8.5	8.6	11.7	6.8
	50	1.8	4.0	3.9	0.6	11.4	3.3	9.5	11.0	4.2	5.1	7.9	10.4	9.0	7.6	7.9	7.8	9.0	6.7
	100	–	3.0	4.5	0	4.1	1.8	8.6	10.4	6.1	5.3	4.1	9.0	5.1	7.1	5.6	6.6	7.7	5.6
	$\bar{X}$	2.1	2.4	3.7	0.6	7.5	2.4	8.9	10.2	6.2	5.4	5.8	9.7	6.6	10.2	10.0	7.1	8.3	
4	0	1.5	2.6	–	0	4.5	2.8	2.7	7.6	3.5	–	5.7	7.4	5.7	8.9	12.0	7.0	7.1	5.3
	5	4.0	2.3	–	0	8.1	3.2	5.6	6.9	4.8	–	7.5	9.0	10.0	16.8	6.7	7.2	7.2	6.6
	10	2.0	0.7	–	0.4	7.7	3.0	8.7	7.6	4.8	–	9.0	8.7	14.8	13.9	7.7	5.9	8.1	6.9
	20	1.3	1.5	–	0.7	6.9	3.4	8.2	5.6	4.9	–	10.7	8.9	6.5	10.3	7.5	5.3	7.0	5.9
	50	0	2.7	–	0.5	3.3	3.6	7.5	7.0	3.8	–	11.6	8.9	5.5	7.2	5.5	5.2	7.7	5.3
	100	0	2.4	–	0.4	5.9	1.5	7.9	4.9	5.2	–	–	9.2	7.8	6.3	4.8	4.0	6.4	4.8
	$\bar{X}$	1.5	2.0		0.3	6.1	2.9	6.8	6.6	4.5		8.9	8.7	8.4	10.6	7.4	5.8	7.3	
5	0	2.5	0.5	–	–	4.0	2.5	8.3	5.1	6.9	–	6.7	3.4	3.4	10.0	4.8	4.7	5.7	4.9
	5	0.5	2.3	–	–	4.4	3.2	8.2	4.9	9.3	–	7.1	3.2	4.4	6.3	5.6	7.7	6.1	5.2
	10	0	3.2	–	–	5.1	3.0	6.0	6.4	9.7	–	7.5	4.6	5.5	9.6	6.9	6.2	4.5	5.6
	20	0	3.2	–	–	0.4	1.8	6.9	4.3	9.0	–	7.9	3.7	8.0	7.6	3.6	6.2	3.1	4.7
	50	0	2.6	–	–	0.4	1.9	8.1	6.2	11.9	–	–	5.3	3.4	2.9	2.0	4.0	2.5	3.9
	$\bar{X}$	0.6	2.4			2.9	2.5	7.5	5.4	9.4		7.3	4.0	4.9	7.3	4.6	5.8	4.4	

nism, *A. carterae*, as the volumes of all samples were doubled by the addition of sea water (31‰), which would bring even the lowest salinity samples within the range of good growth for *A. carterae* (Jitts et al. 1964). Further, runoff water with high silt content was monitored in the lab over a 6-week period and showed no increase in soluble B_{12} concentration during that time.

The release of B_{12} from silt particles into solution, therefore, appears to take place only after it has mixed with sea water. While there was no attempt to define the actual processes involved, Burkholder and Burkholder (1956, 1958) have shown that B_{12} is released from river runoff silt admixed with sea water by microbial activity. This seems to be the case in the Strait of Georgia since existing evidence (Bader et al. 1960) indicates that organic–particle reactions in sea water move toward the formation of complexes rather than the release of organic material from particles into solution.

Thus, the summer peak in B_{12} concentration, as well as the year-round spatial heterogeneity, can be explained by such organic–particle interactions. Throughout much of the year (fall–spring), it may be envisaged that B_{12} is adsorbed onto and released from nonrandomly distributed living and nonliving particulate material, resulting in the patchy distribution observed. Nevertheless, when considering average values of a large number of samples, B_{12} tends to follow the same pattern as nitrate and phosphate because it is also affected by some of the same processes that regulate their seasonal patterns e.g. nutrient uptake, water-column turnover, and associated regenerative processes. During the summer, however, these similarities are completely overshadowed by the introduction of large quantities of particulate matter with river runoff, which results in the summer maximum in B_{12} concentration in the Strait of Georgia.

While the B_{12} particle reaction in the Strait of Georgia may well be determined by additional factors such as *p*H, temperature, and salinity (Bader et al. 1960), the present data suggest that the summer peak of B_{12} is related to particle runoff. Furthermore, the spatial distribution of soluble B_{12} ensuing from this interaction indicates that the annual cycle of B_{12} in coastal waters affected by appreciable amounts of particulate matter is likely to be more complex than has heretofore been supposed.

ANTIA, N. H., C. D. MCALLISTER, T. R. PARSONS, K. STEPHENS, AND J. D. H. STRICKLAND. 1963. Further measurements of primary production using a large-volume plastic sphere. Limnol. Oceanogr. 8: 166–183.

BADER, R. G., D. W. HOOD, AND J. B. SMITH. 1960. Recovery of dissolved organic matter in sea-water and organic sorption by particulate material. Geochim. Cosmochim. Acta 19: 236–243.

BURKHOLDER, P. R., AND L. M. BURKHOLDER. 1956. Vitamin B_{12} in suspended solids and marsh muds collected along the coast of Georgia. Limnol. Oceanogr. 1: 202–208.

1958. Studies on B vitamins in relation to productivity of the Bahia Fosforescente, Puerto Rico. Bull. Mar. Sci. Gulf Carribb. 8: 201–223.

CARLUCCI, A. F. 1970. The ecology of the plankton off La Jolla, California, in the period April through September, 1967. II. Vitamin B_{12}, thiamine, and biotin. Bull. Scripps Inst. Oceanogr. Univ. Calif. 17: 23–31.

1971. Vitamins in waters of Antarctica. Progress report. Research on the marine food chain. Inst. Mar. Resour. Univ. Calif. p. 13–15.

CARLUCCI, A. F., AND P. M. BOWES. 1970a. Production of vitamin B_{12}, thiamine and biotin by phytoplankton. J. Phycol. 6: 351–357.

1970b. Vitamin production and utilization by phytoplankton in mixed culture. J. Phycol. 6: 393–400.

1971a. Distribution of dissolved and particulate vitamin B_{12}, thiamine, and biotin in waters off Southern California. Progress report. Research on the marine food chain. Inst. Mar. Resour. Univ. Calif. p. 16–21.

1971b. Saturation constants for vitamin assimilation of phytoplankton occurring in waters off Southern California. Progress report. Research on the marine food chain. Inst. Mar. Resour. Univ. Calif. p. 57–59.

CARLUCCI, A. F., AND S. B. SILBERNAGEL. 1969. Effect of vitamin concentrations on growth and development of vitamin-requiring algae. J. Phycol. 5: 65–67.

COWEY, C. B. 1956. A preliminary investigation of the variation of vitamin B_{12} in oceanic and coastal waters. J. Mar. Biol. Ass. U.K. 35: 609–620.

DAISLEY, K. W. 1957. Vitamin B_{12} in marine ecology. Nature (London) 180: 1041–1042.

DODIMEAD, A. J., AND G. L. PICKARD. 1967. Annual changes in the oceanic-coastal waters of the eastern subarctic Pacific. J. Fish. Res. Board Can. 24: 2207–2227.

DROOP, M. R. 1954. Cobalamin requirement in Chrysophyceae. Nature (London) 174: 520.

1955. A pelagic marine diatom requiring cobalamin. J. Mar. Biol. Ass. U.K. 34: 228–231.

1957a. Vitamin B_{12} in marine ecology. Nature (London) 180:1041.

1957b. Auxotrophy and organic compounds in the nutrition of marine phytoplankton. J. Gen. Microbiol. 16: 286–293.

1968. Vitamin B_{12} and marine ecology. IV. The kinetics of uptake, growth and inhibition in *Monochrysis lutheri*. J. Mar. Biol. Ass. U.K. 48: 689–733.

DROOP, M. R., J. J. A. MCLAUGHLIN, I. J. PINTNER, AND L. PROVASOLI. 1959. Specificity of some protophytes toward vitamin B_{12}-like compounds. Preprints Int. Oceanogr. Congr. 916–918.

GOLD, K. 1962. Nutrition of marine phytoplankton measured by C^{14} uptake and cell size distribution. J. Protozool. 9: 10 p.

GUILLARD, R. R. L. 1968. B_{12} specificity of marine centric diatoms. J. Phycol. 4: 59–64.

GUILLARD, R. R. L., AND V. CASSIE. 1963. Minimum cyanocobalamin requirements of some marine centric diatoms. Limnol. Oceanogr. 8: 229–239.

JITTS, H. R., C. D. MCALLISTER, K. STEPHENS, AND J. D. H. STRICKLAND. 1964. The cell division rates of some marine phytoplankton as a function of light and temperature. J. Fish. Res. Board Can. 21: 139–157.

LEWIN, R. 1954. A marine *Stichococcus* sp. which requires vitamin B_{12}. J. Gen. Microbiol. 10: 93–96.

MENZEL, D. W., AND J. P. SPAETH. 1962. Occurrence of vitamin B_{12} in the Sargasso Sea. Limnol. Oceanogr. 7: 151–158.

NATARAJAN, K. V. 1970. Distribution and significance of vitamin B_{12} and thiamine in the subarctic Pacific Ocean. Limnol. Oceanogr. 15: 655–659.

PARSONS, T. R., K. STEPHENS, AND R. J. LEBRASSEUR. 1969. Production studies in the Strait of Georgia. Part I. Primary production under the Fraser River plume, February to May, 1967. J. Exp. Mar. Biol. Ecol. 3: 27–38.

PROVASOLI, L. 1963. Organic regulation of phytoplankton fertility, p. 165–219. *In* M. N. Hill, [ed.] The Sea. Vol. 2. Interscience Publishers, Inc. New York, N.Y.

PROVASOLI, L., AND I. J. PINTNER. 1953. Ecological implications of *in vitro* nutritional requirements of algal flagellates. Ann. N.Y. Acad. Sci. 56: 839–851.

RYTHER, J. H., AND R. R. L. GUILLARD. 1962. Studies of marine planktonic diatoms. II. Use of *Cyclotella nana* Hustedt for assays of vitamin B_{12} in seawater. Can. J. Microbiol. 8: 437–445.

STARR, T. J. 1956. Relative amounts of vitamin B_{12} in detritus from oceanic and estuarine environments near Sapelo Island, Georgia. Ecology 37: 658–664.

STRICKLAND, J. D. H., AND T. R. PARSONS. 1965. A manual of sea water analysis. Second edition, revised. Fish. Res. Board Can. Bull. 125: 203 p.

SWEENEY, B. M. 1954. *Gymnodinium spendens*, a marine dinoflagellate requiring vitamin B_{12}. Amer. J. Bot. 41: 821–824.

TULLY, J. P., AND A. J. DODIMEAD. 1957. Properties of the water in the Strait of Georgia, British Columbia, and influencing factors. J. Fish. Res. Board Can. 14: 241–319.

VISHNIAC, H. S., AND G. A. RILEY. 1961. Cobalamin and thiamine in Long Island Sound: patterns of distribution and ecological significance. Limnol. Oceanogr. 6: 36–41.

WALDICHUK, M. 1957. Physical oceanography of the Strait of Georgia, British Columbia. J. Fish. Res. Board Can. 14: 321–486.

WOOD, E. D. F., F. A. J. ARMSTRONG, AND F. A. RICHARDS. 1967. Determination of nitrate in sea water by cadmium-copper reduction to nitrite. J. Mar. Biol. Ass. U.K. 47: 23–31.

Swimming Performance of Young Atlantic Salmon (*Salmo salar*) as Affected by Reduced Ambient Oxygen Concentration

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As oxygen concentration is lowered, a point is reached, the critical oxygen level, at which juvenile Atlantic salmon (*Salmo salar*) stop swimming in a tunnel respirometer. At concentrations just above the critical level, salmon frequently lose position in the upstream end of the respirometer and fall back to the downstream end while still headed into the current. There appears to be a progressive decrease in locomotor activity, indicated by reduced tailbeat frequency, on approaching the critical concentration. Swimming performance of Atlantic salmon is more highly dependent on oxygen concentration than is that of rainbow trout (*Salmo gairdneri*), goldfish (*Carassius auratus*), or *Tilapia*. The critical oxygen level for salmon swimming at 55 cm/sec is about 4.5 mg/liter, in comparison with 2.0 for rainbow trout, 1.7 for goldfish, and 1.0 for *Tilapia*. Between the minimum speed at which a salmon swims steadily and the maximum of which the fish is capable, the critical oxygen level rises with increase in swimming speed. Stoppage of swimming at the critical level is not owing to fatigue; fish resume swimming within minutes of the oxygen level being raised only slightly. The high critical oxygen levels for salmon in relation to other species including rainbow trout, another salmonid, point up the need for maintenance of stringent water quality criteria for oxygen levels in salmon rivers.

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Lorsqu'on abaisse la teneur en oxygène, on en arrive à un point, le niveau critique d'oxygène, où les jeunes saumons atlantiques (*Salmo salar*) cessent de nager dans un respiratomètre à tunnel. A des concentrations juste au-dessus du niveau critique, le poisson perd souvent sa position à l'extrémité d'amont du respiratomètre et retombe à l'extrémité opposée, tout en faisant face au courant. Avec l'approche de la concentration critique, il semble y avoir diminution progressive de l'activité locomotrice, se traduisant par une diminution de fréquence des battements de la queue. La performance à la nage du saumon atlantique est affectée par la teneur en oxygène à un degré beaucoup plus grand que celle de la truite arc-en-ciel (*Salmo gairdneri*), du cyprin doré (*Carassius auratus*), ou de *Tilapia*. Le niveau critique d'oxygéne pour une nage de 55 cm/sec est *ca* 4.5 mg/litre pour le saumon, comparativement à 2.0 pour la truite arc-en-ciel, 1.7 pour le cyprin doré et 1.0 pour *Tilapia*. Entre la vitesse minimale de nage soutenue du saumon et la vitesse maximale dont il est capable, le niveau critique d'oxygène augmente à mesure qu'augmente la vitesse de nage. Ce n'est pas la fatigue qui cause l'arrêt de la nage; le poisson recommence à nager en moins de quelques minutes après une élévation, même minime, du niveau d'oxygène. Les niveaux critiques élevés chez le saumon, comparativement à d'autres espèces, y compris la truite arc-en-ciel, un autre salmonoïde, font ressortir l'importance du maintien de rigoureux critères de qualité de l'eau en vue d'assurer des niveaux adéquats d'oxygène dans les rivières à saumons.

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THE influence of ambient oxygen on the swimming performance of fishes varies among species (Kutty 1968a; Pandian 1969; Doudoroff and Shumway 1970). Kutty observed that, at comparable speeds, goldfish (*Carassius auratus*) continue swimming at much lower oxygen concentrations than do rainbow trout (*Salmo gairdneri*). Pandian found that oxygen dependence of swimming activity in the freshwater fishes, *Tilapia mossambica*, *Rasbora daniconius*, *Puntius stigma*, and *Neuria danrica*, increased in the order listed. Davis et al. (1963) report that the oxygen dependence of swimming in chinook salmon (*Oncorhynchus tshawytscha*) is greater than in coho (*O. kisutch*). The work reported here was done to provide information on the swimming performance of Atlantic salmon (*Salmo salar*) in relation to environmental oxygen levels.

Materials and Methods

Hatchery-reared Atlantic salmon smolts of mean total length 23.4 cm (range, 21.5–24.7 cm) and mean weight 110.3 g (range, 87–135 g) were held in flowing water in large, cylindrical fiberglass tanks. Separate groups were held in fresh water with natural photoperiod, sea water with natural photoperiod, and fresh water with reciprocal photoperiod as described by Saunders and Henderson (1970). The main emphasis was placed on the natural photoperiod, freshwater group; the other groups provided additional, tentative information on effects of photoperiod and salinity. Fish were acclimated at 15 C during May–July, 1971. Experiments were done at 15 C during June and July.

The apparatus used was an improved model of Blazka's respirometer (Blazka et al. 1960; Smit 1965; Smith and Newcomb 1970) of 41-liter capacity, having a swimming tunnel 108 cm long and 12 cm in diameter. The upstream two-thirds of the tunnel was partially covered with black polyethylene plastic to reduce visual disturbance of the fish. The downstream one-third was left uncovered to allow the observers to see when the fish fell back against a barrier screen. The fish were starved for 24 hr before being placed in the respirometer. Following a brief training period immediately after the fish were put in the chamber, they were left undisturbed overnight before experiments.

The experimental procedure was that followed by Kutty (1968a), except that in our experiments dissolved oxygen in the ambient water was reduced by bubbling nitrogen through a diffuser immersed in a top well of the respirometer. Water flow through the respirometer was maintained at 750 ml/min unless otherwise stated. The fish was forced to swim at a fixed speed, often for 1–2 hr, at oxygen levels near air saturation. Then the oxygen concentration was lowered rapidly to a point above the critical level judged from preliminary tests as the concentration at which the fish stops swimming (Fig. 1). Below this point the oxygen level was reduced slowly at a rate of 0.02–0.04 mg/liter/min. As oxygen concentration approached the critical level, the fish repeatedly lost its position in the upstream end of the swimming tunnel and lagged to the downstream end while still headed into the water current. This is called a "reverse." The number of reverses increased toward the approach of the critical oxygen level (Fig. 1). With further reduction of oxygen, the fish fell back and its tail briefly touched the grid at the downstream end and these grid touches usually became more frequent. The critical level was taken as the mean oxygen concentration at the times of the first two grid touches. Finally, the fish stopped swimming and rested with its tail touching the grid. After one or two such stops, the fish often collapsed and was swept sideways against the grid, especially at high water velocities. At low velocities the fish usually stopped with its tail against the grid and with complete cessation of tail beats but without curling up on the grid.

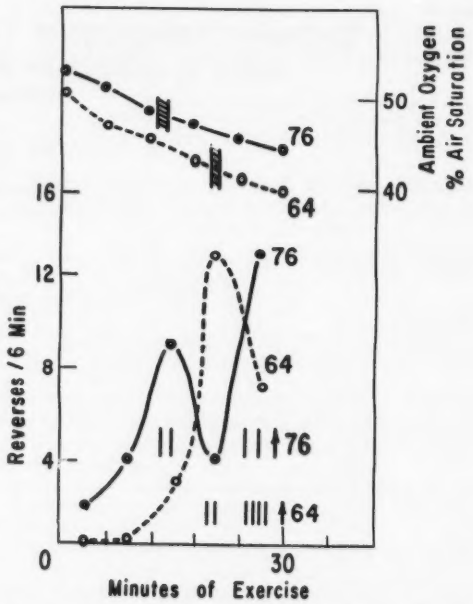

FIG. 1. Swimming behavior of a 107-g Atlantic salmon (*Salmo salar*) smolt during gradual reduction of ambient oxygen in a Blazka respirometer at two current velocities (64 and 76 cm/sec). Trends in ambient oxygen (upper curves) and "reverses" by the fish (lower curves) are indicated. Vertical lines at the bottom of the figure denote the times when the fish touched the metal grid at the downstream end of the swimming tunnel. Arrows indicate collapse of the fish. The hatched areas on the upper curves indicate the critical oxygen levels for the respective swimming speeds. In each case the fish swam steadily for 2 hr at the respective speeds immediately before the events shown in the figure.

Experiments were done under conditions of salinity and temperature of acclimation. No attempt was made to continue photoperiod regulation during experiments. The

fish were made to swim at water current velocities of 49, 55, 64, 70, and 76 cm/sec, speeds at which they could swim for several hours. Salmon smolts did not swim well at current velocities lower than 49 cm/sec. In contrast with species tested earlier (Kutty 1968a; Pandian 1969), salmon often held position using their large pectoral fins on the bottom of the swimming tunnel at these low speeds. The metal grid at the downstream end of the tunnel was electrified only while training the fish. Salmon smolts were more excitable than rainbow trout and goldfish (Kutty 1968a). Results presented here are from fish of proven ability to swim continuously for some hours in the respirometer at moderate current velocities. Dissolved oxygen concentration was either monitored continuously using a Beckman Model 160 Physiological gas analyzer with an oxygen electrode or at intervals using a WPRL dropping mercury electrode (Southern Analytical, Surrey, U.K.).

Results and Discussion

The results indicate that the swimming performance of salmon smolts is highly dependent on ambient oxygen (Fig. 2). At the highest speed used (76 cm/sec, equivalent to about 3.2 body lengths/sec), the critical oxygen level (resulting in stoppage of swimming) was 5 mg/liter, about 50% air saturation and, at the lowest speed (49 cm/sec or about 2.1 body lengths/sec), it was 4 mg/liter. Since 76 cm/sec was near the maximum swimming speed of the salmon at oxygen levels close to air saturation under the conditions of our experiment, the curve can be expected to be asymptotic at the upper end. The lower end of the curve was limited by the minimum speed at which we were able to keep the fish swimming steadily.

The curve for salmon in Fig. 2 is fitted by eye through the mean values for fish from fresh water with natural photoperiod. Salmon from sea water with natural photoperiod and fresh water with reciprocal photoperiod behaved little differently from those in fresh water with natural photoperiod. Values for the seawater group lie more to the left and indicate less dependence on oxygen for swimming than the freshwater groups. However, no attempt is made here to distinguish effects of photoperiod and salinity. The combined data (Fig. 2) show clearly that the swimming performance is influenced more by reduced oxygen levels in salmon than in goldfish, rainbow trout, and *T. mossambica*.

Davis et al. (1963) found that swimming performance of juvenile chinook and coho salmon was dependent on oxygen at all levels below air saturation but this was especially evident at levels below 50% of air saturation. Graham (1949) observed that oxygen limited swimming performance of brook trout (*Salvelinus fontinalis*) at levels below 6 mg/liter at 8 C. In the yellow perch (*Perca flavescens*),

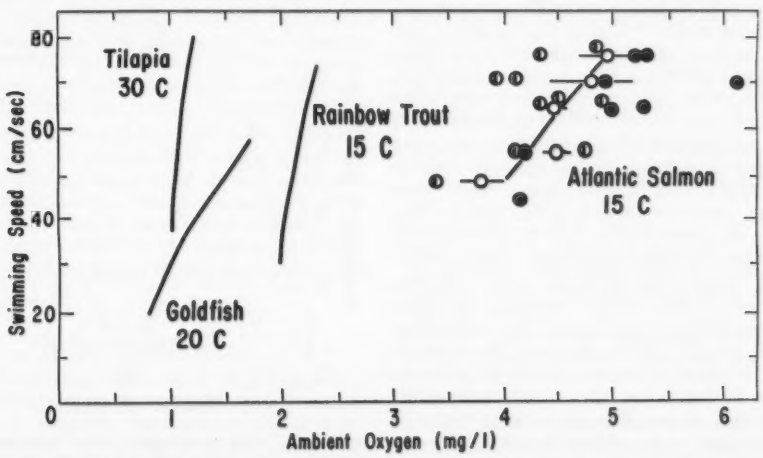

FIG. 2. Swimming performance of Atlantic salmon smolts in relation to ambient oxygen concentration compared with data for rainbow trout (*Salmo gairdneri*), goldfish (*Carassius auratus*), and *Tilapia mossambica*. The salmon curve is for fish acclimated to fresh water and natural photoperiod (open circles) and shows the mean and one standard error for 4–5 determinations of critical oxygen level at each of five swimming speeds. Individual critical oxygen concentrations for smolts in fresh water with reciprocal photoperiod (closed circles) and in sea water with natural photoperiod (half-closed circles) are shown for comparison to fresh water with natural photoperiod.

Ferguson (1957) found that swimming performance was clearly limited by oxygen levels below 3 mg/liter at 15 C with higher oxygen concentrations having some influence on swimming speed.

Ferguson (1957), Davis et al. (1963), Smit et al. (1971), and Jones (1971) showed an effect of temperature on the swimming performance in relation to oxygen level in yellow perch, Pacific salmon, goldfish, and rainbow trout, respectively. The observations on various fishes shown in Fig. 2 were obtained from fishes of approximately equal size at temperatures at which each species was optimally active. The critical oxygen concentration for Atlantic salmon swimming at 55 cm/sec is about 4.5 mg/liter, in comparison with 2.0 for rainbow trout, 1.7 for goldfish, and 1.0 for *Tilapia*. Atlantic salmon have a performance curve far to the right of the rainbow trout, another salmonid. While the anaerobic abilities of Atlantic salmon are little known, the rainbow trout does have slight anaerobic ability (Kutty 1968b). It is of interest in this connection that Davis et al. (1963) found that the performance curve for chinook salmon used a second time in the activity chamber (i.e., trained) was much steeper and farther to the left than that for fish during their first trial.

Failure of salmon to maintain sustained swimming speeds at low ambient oxygen levels is not always owing to fatigue. If such fish were fatigued, they would be unable to resume swimming, as they did, within minutes of the oxygen level being raised only slightly. In salmon, as in other species (Kutty 1968a; Smit 1965), immediately after a swimming failure, the fish swam well at the same speed at which they failed if the oxygen concentration was raised slightly above the critical level for the particular swimming speed. While lack of motivation has been cited as the cause of failure at high swimming speeds (Smit et al. 1971), failure to swim at lower speeds under critical oxygen conditions has been cited as resulting from a behavioral reflex mechanism (Kutty 1968a). How this mechanism operates, either through peripheral or central oxygen receptors (Saunders and Sutterlin 1971), is not known. The mechanism is probably not sensing an excess accumulation of lactic acid in the muscles or in the blood since the resumption of swimming after a failure is immediate, provided ambient oxygen concentration is above the critical level as shown in goldfish (Kutty 1968a; Smit 1965), rainbow trout (Kutty 1968a), and, now, Atlantic salmon.

There appears to be a progressive decrease in locomotor activity of the fish at the approach of a critical oxygen concentration. As an index of motor activity of the locomotor muscles, we counted the number of times the fish allowed itself to be swept downstream (reverses) in the swimming tunnel while oxygen was gradually lowered. Since each reverse occurs consequent to a reduction in tail beat frequency (TBF), each such reverse can be taken as owing to a reduction in motor activity of the locomotor muscles. Each reverse was often quickly followed by a forward thrust. Thus, it appeared that, if a reduction in TBF resulted in a reverse, motor activity showed a subsequent increase each time to keep the fish in position upstream, thereby indicating that motivation may be an important factor inducing the fish to continue swimming (Smit et al. 1971). However, swimming failure occurs abruptly. The tail touches the grid at the downstream end of the tunnel (Fig. 1) but the fish usually darts forward immediately after the first such encounter. Falling back onto the grid and frequent reverses are the first clear indications of the approach of the critical oxygen concentration. This behavior pattern was similar in goldfish, rainbow trout, *Tilapia*, and salmon but occurred at different ambient oxygen concentrations. However, grid touching by salmon

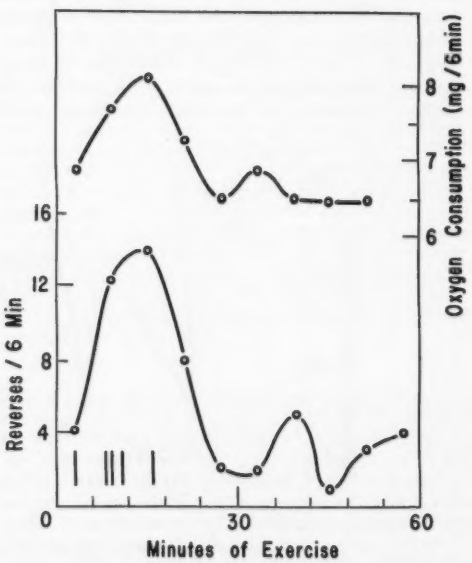

FIG. 3. Trends of metabolic rate, "reverses," and "grid touches" of a 129-g Atlantic salmon smolt during the initial hour of swimming at a current velocity of 76 cm/sec in a closed respirometer. Ambient oxygen did not fall below 70% air saturation. The fish overcame the initial stress response signified by the increased metabolic rate, reverses, and grid touches, and continued swimming steadily for another 2 hr. Vertical lines indicate grid touches.

was followed more quickly by collapse and curling up on the grid. Thus, it appears that the mechanism responsible for the decrease in motor activity of the locomotor muscles at sustained speeds is operative in Atlantic salmon at much higher ambient oxygen levels than in other species studied.

Swimming failures resulting from high effort at high swimming speeds and from low oxygen concentration appeared to be similar from the behavior of the fish in the respirometer. In both, frequent reverses and grid touches were followed by collapse. When the salmon is forced to accelerate quickly to high speeds (e.g. from 0 to 76 cm/sec), even when no failure results, there are usually frequent reverses and occasional grid touching (Fig. 3). Salmon often recover from this stress and swim steadily thereafter for several hours. If the fish is not able to overcome the initial stress of imposed activity at high speeds, it collapses. In a typical example of failure owing to high effort, a salmon smolt collapsed after 6 min at a speed of 87 cm/sec when it curled up on the grid after 19 reverses and 10 grid touches. The initial stress owing to high swimming effort is also noticeable from the trend of metabolic rates (Fig. 3), which suggests wastage of energy during irregular swimming (Brett 1964; Smit 1965; Kutty 1968b; Kutty 1969).

Water velocity and dissolved oxygen concentration are important ecological factors in the distribution and success of stream dwelling salmonids. Atlantic salmon are well known for their ability to negotiate swift rivers and waterfalls *en route* to their spawning grounds. Our work with salmon smolts shows that the oxygen requirements for swimming performance of this species are stringent. There is a need to confirm this oxygen sensitivity in adult salmon moving upriver to their spawning grounds. Water quality criteria for oxygen levels in salmon rivers need to be stringent indeed if the number of suitable rivers for this endangered species is not to be further reduced through man's demands on rivers.

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BLAZKA, P., M. WOLF, AND M. CEPELA. 1960. A new type of respirometer for the determination of the metabolism of fish in the active state. Physiol. Bohemoslov. 9: 553–559.

BRETT, J. R. 1964. The respiratory metabolism and swimming performance of young sockeye salmon. J. Fish. Res. Board Can. 21: 1183–1226.

DAVIS, G. E., J. FOSTER, C. E. WARREN, AND P. DOUDOROFF. 1963. The influence of oxygen concentration on the swimming performance of juvenile Pacific salmon at various temperatures. Trans. Amer. Fish. Soc. 92: 111–124.

DOUDOROFF, P., AND D. L. SHUMWAY. 1970. Dissolved oxygen requirements of freshwater fishes. FIRI/T86 Water Pollution. FAO Tech. Pap. 86: 291 p.

FERGUSON, R. G. 1957. Unpublished data cited by F. E. J. Fry in The aquatic respiration of fish, p. 1–63. *In* M. E. Brown [ed.] Physiology of fishes. Vol. I. Academic Press, Inc., New York, N.Y.

GRAHAM, J. M. 1949. Some effects of temperature and oxygen pressure on the metabolism and activity of the speckled trout, *Salvelinus fontinalis*. Can. J. Res. D27: 270–288.

JONES, D. R. 1971. The effect of hypoxia and anaemia on the swimming performance of rainbow trout (*Salmo gairdneri*). J. Exp. Biol. 55: 541–551.

KUTTY, M. N. 1968a. Influence of ambient oxygen on the swimming performance of goldfish and rainbow trout. Can. J. Zool. 46: 647–653.

1968b. Respiratory quotients in goldfish and rainbow trout. J. Fish. Res. Board Can. 25: 1689–1728.

1969. Oxygen consumption in the mullet (*Liza macrolepis*) with special reference to swimming velocity. Mar. Biol. 4: 239–242.

PANDIAN, K. J. 1969. Influence of dissolved oxygen on the swimming performance of some South Indian freshwater fishes. M. Phil. Thesis. Madurai University, Madurai, India.

SAUNDERS, R. L., AND E. B. HENDERSON. 1970. Influence of photoperiod on smolt development and growth of Atlantic salmon (*Salmo salar*). J. Fish. Res. Board Can. 27: 1295–1311.

SAUNDERS, R. L., AND A. M. SUTTERLIN. 1971. Cardiac and respiratory responses to hypoxia in the sea raven, *Hemitripterus americanus*, and an investigation of possible control mechanisms. J. Fish. Res. Board Can. 28: 491–503.

SMIT, H. 1965. Some experiments on the oxygen consumption of goldfish (*Carassius auratus* L.) in relation to swimming speed. Can. J. Zool. 43: 623–633.

SMIT, H., J. M. AMELINK-KOUTSTAAL, J. VIJVERBERG, AND J. C. VON VAUPEL-KLEIN. 1971. Oxygen consumption and efficiency of swimming goldfish. Comp. Biochem. Physiol. 39A: 1–28.

SMITH, L. S., AND T. W. NEWCOMB. 1970. A modified version of the Blazka respirometer and exercise chamber for large fish. J. Fish. Res. Board Can. 27: 1321–1324.

Choice of Colors of Food Items by Rainbow Trout (*Salmo gairdneri*)

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Rainbow trout in artificial stream situations were offered seven colors of dyed rainbow trout eggs in all combinations of two colors. Against a pale, greenish-blue background, in daylight, the color preference was: blue, red, black, orange, brown, yellow, and green. This order largely reflected contrast with background color; but, in addition, there were preferences for certain colors. Particular color combinations such as yellow and black, and yellow and blue, resulted in significantly higher total consumption. When background color was varied, preference was low for the color of eggs that matched the background, but the effect was not statistically demonstrable. At low light intensities the order of preference for various colors was yellow, red, blue, and black. The results collectively indicate keen color discrimination in rainbow trout and preferences for particular colors in particular environmental settings. These findings could have useful application to field situations where it is desirable to modify rates of predation on salmon fry by presenting other kinds of food to satiate predators.

GINETZ, R. M., AND P. A. LARKIN. 1973. Choice of colors of food items by rainbow trout (*Salmo gairdneri*). J. Fish. Res. Board Can. 30: 229–234.

On a offert à des truites arc-en-ciel en milieu artificiel à eau courante des oeufs de truite arc-en-ciel séchés et colorés de sept teintes différentes, dans toutes les combinaisons possibles de deux couleurs. Sur fond pâle, bleu verdâtre, à la lumière du jour, les couleurs préférées sont, par ordre: bleu, rouge, noir, orange, brun, jaune et vert. Cet ordre de préférence réflète en grande partie le degré de contraste avec la couleur de fond; toutefois, il y a préférence plus marquée pour certaines couleurs. Des combinaisons particulières de couleurs, jaune et noir ou jaune et bleu, par exemple, ont pour effet une consommation totale nettement supérieure. Si on modifie la couleur de fond, la préférence est peu marquée pour des oeufs dont la couleur s'appareille à celle de fond; l'effet ne peut cependant pas être prouvé statistiquement. A de faibles intensités lumineuses, l'ordre de préférence des diverses couleurs est: jaune, rouge, bleu et noir. Ces résultats, considérés collectivement, démontrent une fine discrimination des couleurs chez la truite arc-en-ciel, et une préférence marquée pour une couleur particulière dans un milieu donné. Ces observations peuvent s'appliquer avec profit à des situations dans la nature, où il est désirable de modifier le taux de prédation sur les alevins de saumon en présentant d'autres types de nourriture aux prédateurs pour les rassasier.

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IN the course of investigations on factors influencing rainbow trout (*Salmo gairdneri*) predation on sockeye salmon (*Oncorhynchus nerka*) fry, the possibility was raised that trout may tend to feed more on some colors of food items in particular environmental settings. This paper describes experiments in which eggs of rainbow trout were dyed various colors and offered in different combinations of two colors, in experimental streams, to rainbow trout juveniles (about 25-cm fork length). The color of the bottom of the stream was varied in a second series of observations, and light intensity in a third series. The colors of food items and backgrounds in the experiments had no particular significance to natural situations, but merely provided an apparently suitable set of experimental conditions for demonstration of potential for differential predation on different colored food items.

Materials and Methods

The study was conducted at the trout hatchery of the British Columbia Fish and Wildlife Branch near Abbotsford. Four troughs, each 7.3 × 0.6 × 0.5 m, constructed

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of plywood of 1.6-cm thickness, were suspended in Marshall Creek, British Columbia, so as to maintain a water depth of 30 cm. Water velocity was regulated by wooden gates at 38 ± 2 cm/sec.

Rainbow trout used as predators were 2-year-olds of hatchery domestic stock, and were almost uniformly 25-cm fork length. Their previous diet was pelleted hatchery food of reddish brown color. Five trout were placed in each trough, where they were retained for the duration of the experiment. Prey were dead rainbow trout eggs from the Fish and Wildlife Branch hatchery at Summerland, B.C. Eggs that were not eaten were collected in a trap of No. 5 nylon mesh suspended at the outlet end. Eggs were colored by 48-hr immersion in proprietary fabric dyes (350 ml of water and 0.5-g dye). Immediately prior to a trial feeding, the eggs were washed in 50 ml of water to remove surface dye.

Prior to each trial, the troughs were cleared of silt and debris. The time between trials was 24 hr, and the trout were not fed between trials. All trials were conducted at 1000 hr and lasted for 10 min. In the first and second experiments, the light source was natural and the intensity ranged from 330 to 380 ft-c. In the third experiment, the light source was artificial and could be varied. The troughs were enclosed in chambers made of plywood and 4-ml black polyethylene. Twelve 7.5 watt General Electric incandescent light bulbs were suspended above each trough and controlled by a Variac. Light intensities at the water surface were measured with a Gossen foot candle meter (Trilux Model) fitted with a scotopic correction filter. Spectral characteristics of the light source were variable; reds predominated at low intensities.

In the first and third series of trials, the troughs were pale greenish-blue (turquoise). In the second series, the bottom was colored red, yellow, dark blue, or black, and approximately matched with particular colors of eggs.

In each trial 35 eggs of each of two colors were placed in the inlet of a trough. In the first and third experiments the color combinations were all possible in the series: red, blue, black, brown, green, yellow, orange (21 combinations); plus each color with itself (70 eggs), for a total of 28 trials in a complete series. In the first experiment, the complete series was replicated four times in a randomized block design. In the second experiment, four egg colors were used: blue, red, black, and yellow; and there was only one replication. In the third experiment, the same four colors were used and there were two replicates used in a randomized block design.

In each case the analysis conforms to standard procedure for factorial experiments. For example, in the data of Table 1 the column sums measure the preference shown for a particular color, while the row sums indicate the effect of a color on consumption of other colors. The interaction of column and row effects measures the degree to which particular color combinations have synergic effects. In all tests of significance, $\alpha = 0.05$.

Results

With natural light and a pale greenish-blue background, the number of eggs of various colors eaten was significantly different. The colors were chosen in the order of preference: blue, red, black, orange, brown, yellow, and green (Table 1). The effect of a color in eliciting a high consumption of eggs of other colors was also significant, being highest for yellow, followed by green, brown, orange, black, blue, and red. Because the eggs were fed in combinations of two colors, the row and column effects were in some measure interdependent and, hence, show the colors in nearly reverse sequence. For example, if the total consumption of eggs per trial tended to be constant, and if one color of a pair was strongly preferred, then the second color would appear to have "caused" the high consumption of the first. However, this was not entirely the explanation because, statistically, the interaction was also significant. Certain color combinations were associated with greater total consumption. For example, yellow and black in combination resulted in high total consumption, and this was also true of yellow and blue, and of red and orange. Conversely, yellow with orange gave especially low total consumption, and this was also true of green with red.

It is also noteworthy that the results were remarkably consistent. In the 84 trials involving two different colors, 69 gave results that were consistent with the preference hierarchy indicated by the column sums; 9 showed reversals (only of minor proportions); and 6 resulted in ties. The trout were evidently capable of making highly consistent choices between particular colors. This was rather surprising considering that the spread in total consumption of the various colors was only 827–570.

In the second series of experiments, four background colors were matched as closely as possible to four egg colors (blue, red, black, and yellow), and 35 eggs of each of two colors were offered in all possible combinations, including each color with itself. Except for the blue background, consumption of the egg color that matched the background was low (Table 2). For the red background, the consumption of yellow was highest, and vice versa. For the black background, consumption of red was highest, closely followed by yellow. These results suggest the importance of contrast with the background color as a factor influencing choice. However, this was not true of the set of trials involving a blue background, in which the consumption of blue eggs was highest, closely followed by red and yellow.

In analysis of variance of the data of Table 2, there was no statistically significant interaction between color preference and background. There were significant differences between the numbers of eggs eaten of various colors, which were attributable to the small number of black eggs consumed (Table 2,

TABLE 1. Number of eggs of various colors eaten by trout in each four replicates when eggs were offered in all possible combinations of two colors. Seventy eggs were offered in each trial, 35 of each of two colors. When each of the two colors was the same, the figure recorded is one-half the total number eaten. Background color of sides of trough was pale blue. Replicates are in vertical order and their sum is indicated in each cell by the number below the line.

Second color	No. eggs of a color, eaten in the presence of a second color							Replicate totals	Row totals
	Blue	Red	Orange	Green	Brown	Black	Yellow		
Blue	31.0	26.0	26.0	7.0	22.0	20.0	34.0	166.0	
	33.5	28.0	30.0	16.0	27.0	21.0	27.0	182.5	
	28.5	30.0	26.0	24.0	12.0	26.0	24.0	170.5	668.0
	24.0	26.0	19.0	15.0	22.0	26.0	17.0	149.0	
	117.0	110.0	101.0	62.0	83.0	93.0	102.0		
Red	24.0	29.0	27.0	5.0	15.0	25.0	4.0	129.0	
	22.0	28.5	28.0	12.0	22.0	14.0	29.0	155.5	
	34.0	26.5	23.0	13.0	23.0	21.0	20.0	160.5	588.5
	21.0	21.5	25.0	16.0	22.0	16.0	22.0	143.5	
	101.0	105.5	103.0	46.0	82.0	76.0	75.0		
Orange	33.0	32.0	20.0	18.0	16.0	18.0	16.0	153.0	
	35.0	31.0	29.0	20.0	25.0	32.0	21.0	193.0	
	30.0	31.0	16.5	26.0	18.0	29.0	16.0	166.5	682.5
	21.0	25.0	31.0	28.0	26.0	25.0	14.0	170.0	
	119.0	119.0	96.5	92.0	85.0	114.0	67.0		
Green	34.0	23.0	30.0	25.5	33.0	33.0	14.0	192.5	
	34.0	23.0	20.0	19.5	35.0	33.0	25.0	189.5	
	24.0	22.0	32.0	26.0	22.0	29.0	35.0	190.0	744.0
	27.0	33.0	23.0	13.0	30.0	33.0	13.0	172.0	
	119.0	101.0	105.0	84.0	120.0	128.0	87.0		
Brown	31.0	22.0	23.0	23.0	23.0	34.0	28.0	184.0	
	33.0	27.0	25.0	34.0	21.5	18.0	27.0	185.5	
	20.0	26.0	25.0	16.0	26.5	33.0	26.0	172.5	705.0
	23.0	29.0	21.0	26.0	24.0	22.0	18.0	163.0	
	107.0	104.0	94.0	99.0	95.0	107.0	99.0		
Black	27.0	25.0	26.0	17.0	22.0	18.0	29.0	164.0	
	33.0	31.0	24.0	25.0	11.0	33.0	21.0	178.0	
	35.0	16.0	22.0	14.0	33.0	33.0	34.0	187.0	671.5
	33.0	21.0	12.0	22.0	10.0	21.5	23.0	142.5	
	128.0	93.0	84.0	78.0	76.0	105.5	107.0		
Yellow	35.0	31.0	24.0	28.0	35.0	35.0	17.5	205.5	
	35.0	35.0	35.0	25.0	34.0	34.0	23.5	221.5	
	35.0	33.0	25.0	29.0	27.0	35.0	31.5	215.5	800.0
	31.0	26.0	0	27.0	20.0	32.0	21.5	157.5	
	136.0	125.0	84.0	108.0	116.0	136.0	94.0		
Replicate totals	215.0	188.0	176.0	123.5	166.0	183.0	142.5	1194.0	
	225.5	203.5	191.0	151.5	175.5	185.0	173.5	1305.5	
	206.5	184.5	169.5	148.0	161.5	206.0	186.5	1262.5	
	180.0	181.5	131.0	147.0	154.0	175.5	128.5	1097.5	
Column totals	827.0	757.5	667.5	570.0	657.0	749.5	631.0	Grand total	4859.5

Total). There were also significant differences in the consumption with various backgrounds, with least total consumption on the black background, and most on the blue. There were no other significant differences.

The third series of experiments offered two color combinations from the four colors (blue, red, black, and yellow) at four low light intensities (0.1, 0.4, 0.8, and 1.2 ft-c) with the same pale, greenish-blue background as the first series of experiments. The entire set was replicated twice. The results (Table 3) reinforce the conclusions of the first experiment in indicating consistent preferences for particular colors. Within each light intensity, the results of individual trials coincided with the preference indicated by the column totals in 33 of the total of 48 trials. In contrast to the results of the first experiment (Table 1), in which, at 330 ft-c light intensity, the preference was in the order blue, red, black, and yellow, at the low light intensities the preference in the aggregate was yellow, red, blue, and black. Presumably, when there was a low light intensity from an artificial source, the yellow and red provided a greater contrast when the illumination was from above. The mean consumption of eggs per trial was 16.63 compared to 24.80 in the first experiment, indicating a decreased efficiency of capture at lower light levels. The difference was not similar for the various colors; for example, the average consumption of blue per trial was less than half as many eggs at the lower light levels (14.64 compared to 29.54); the consumption of yellow was almost the same (20.33 compared to 22.54).

Analysis of variance indicated significant differences in all main effects i.e. greater consumption at higher levels of light, differences in consumption of various colors, and difference in total consumption of other colors when a particular color was fed

TABLE 2. Number of eggs of various colors eaten by trout in troughs with background colors matching egg colors. Seventy eggs were offered in each trial, 35 of each of two colors. When each of the two colors was the same, the figure recorded is one-half the total number eaten.

Background color	Second color	No. eggs of a color, eaten in the presence of a second color				
		Blue	Red	Black	Yellow	Totals
Blue	blue	28.0	26.0	27.0	30.0	111.0
	red	21.0	34.0	10.0	22.0	87.0
	black	33.0	24.0	17.5	16.0	90.5
	yellow	25.0	17.0	22.0	33.0	97.0
	Totals	107.0	101.0	76.5	101.0	385.5
Red	blue	24.5	22.0	4.0	15.0	65.5
	red	27.0	7.5	19.0	33.0	86.5
	black	11.0	24.0	19.5	35.0	89.5
	yellow	30.0	11.0	17.0	21.0	79.0
	Totals	92.5	64.5	59.5	104.0	320.5
Black	blue	17.0	20.0	13.0	18.0	68.0
	red	10.0	20.5	11.0	25.0	66.5
	black	16.0	24.0	9.0	14.0	63.0
	yellow	11.0	28.0	9.0	16.0	64.0
	Totals	54.0	92.5	42.0	73.0	261.5
Yellow	blue	26.5	19.0	21.0	8.0	74.5
	red	9.0	24.0	14.0	11.0	58.0
	black	19.0	24.0	18.0	24.0	85.0
	yellow	18.0	26.0	20.0	25.5	89.5
	Totals	72.5	93.0	73.0	68.5	307.0
Total	blue	96.0	87.0	65.0	71.0	319.0
	red	67.0	86.0	54.0	91.0	298.0
	black	79.0	96.0	64.0	89.0	328.0
	yellow	84.0	82.0	68.0	95.5	329.5
	Totals	326.0	351.0	251.0	346.5	1274.5

TABLE 3. Number of eggs of various colors eaten by trout at four different light intensities in each of two replicates when eggs were offered in all possible combinations of two colors. When each of two colors was the same, the figure recorded is one-half the total number eaten. Background color of trough was pale blue. Replicates are in vertical order.

Second color	No. eggs of a color, eaten in the presence of a second color				
	Blue	Red	Black	Yellow	Totals
	Light intensity of 0.1 ft-c				
Blue	4	14	2	14	65
	0	11	3	17	
Red	6	3	3	9	39.5
	7	3.5	1	7	
Black	2	16	2	14	65.5
	2	11	2.5	16	
Yellow	7	12	23	10	87.5
	2	2	23	8.5	
Totals	30	72.5	59.5	95.5	257.5
	Light intensity of 0.4 ft-c				
Blue	9	20	10	25	123.5
	10.5	19	5	25	
Red	6	15	9	10	72.5
	7	8.5	4	13	
Black	22	19	8	25	127.5
	11	12	8.5	22	
Yellow	22	14	29	15.5	160.5
	18	16	28	18	
Totals	105.5	123.5	101.5	153.5	484
	Light intensity 0.8 ft-c				
Blue	17.5	21	12	28	160.5
	15	29	16	22	
Red	13	23	10	16	118
	18	15	6	17	
Black	30	32	13	23	181
	17	26	15	25	
Yellow	21	16	32	17.5	183
	21	25	31	19.5	
Totals	152.5	187	135	168	642.5
	Light intensity 1.2 ft-c				
Blue	24.5	25	13	29	174.5
	20	27	10	26	
Red	15	27	15	29	163.5
	17	20.5	6	34	
Black	29	32	15	25	194.5
	24	24	19.5	26	
Yellow	25	30	31	34	248.5
	26	29	43	30.5	
Totals	180.5	214.5	152.5	233.5	781
	Totals				Grand totals
Blue	100.5	166	71	186	523.5
Red	89	115.5	54	135	393.5
Black	137	172	83.5	176	568.5
Yellow	142	144	203	153.5	642.5
Grand totals	468.5	597.5	411.5	650.5	2128

with them. The last-mentioned effect, as in the first experiment, was attributable to the effect of yellow in enhancing consumption of other colors, and the reverse effect of red. The interaction of row and column effects was also significant and was apparently related to the particular synergic effects of yellow and black in combination, and yellow and blue in combination.

The interaction between color (column effect) and light intensity was also significant, and reflected the minor differences in the hierarchy of color preference at different light intensities. The four light intensities were apparently not sufficiently different to produce any major pattern of change in color preference.

Finally, the interaction between light intensity and effect of color in enhancing consumption (row effect) was not significant, nor was the second order interaction of row effect, column effect, and light intensity.

Discussion

The foregoing experiments indicate that, given a choice of two kinds of food items that are identical, except for color, rainbow trout exercise consistent preferences for one color in relation to another. Some of this preference is explicable by the contrast of a color with the background color. An added element in choice of color seems to be related to the color per se. At high light intensities blue and red were the most preferred. Blue was also preferred against a blue background, suggesting that it may perhaps have some particular attraction. At low light intensities consumption was highest for yellow, even though the background was pale greenish-blue. It is to be noted that the light intensities used (0.1–1.2 ft-c) are in the range in which color vision of sockeye salmon is marginally operative (Ali 1959). In these circumstances, contrast may be much more important than color in influencing color selection.

In addition to these major effects, it was indicated that the presence of some colors enhances the consumption of others. For example, consumption of other colors was highest in the presence of yellow at both high and low light intensities. Additionally, particular color combinations give extra enhancement. For example, yellow and black together led to higher consumption of both colors at both high and low light intensities.

The particular results of these experiments may be applicable only to rainbow trout of a particular situation with a prior hatchery experience. Nevertheless, they indicate a capacity for color discrimination that could have significance to the rate of

predation on prey of various colors and to the relative attractiveness of various kinds of artificial lures in particular circumstances. Hurst (1953) came to a similar conclusion regarding bluegills (*Lepomis macrochirus*). Greater rates of trout predation on sticklebacks with red markings were observed by Moodie (1972).

Predation on migrating salmon fry by trout (and other species of fish) might be reduced by providing alternate foods before the time of the fry migration. Because of the slow rate at which they are digested, salmon eggs are a good possible alternate food. The foregoing experiments indicate that trials with eggs dyed different colors might lead to the use of colored eggs that would result in higher rates of consumption by trout. Thus, if the particular color preferences shown in the present series of experiments had wide applicability, it would be useful to put blue (or black) and yellow eggs into a stream in the late afternoon hours. In these circumstances, the trout would be given the greatest "main course" of eggs, and would presumably be less inclined to a large "dessert" of fry from the evening fry migration.

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ALI, M. A. 1959. The ocular structure, retinomotor and photobehavioral responses of juvenile Pacific salmon. Can. J. Zool. 37: 965–996.

HURST, P. M., JR. 1953. Can fish see color? Progr. Fish-Cult. 15 (3): 95.

MOODIE, G. E. E. 1972. Predation, natural selection and adaptation in an unusual threespine stickleback. Heredity 28: 155–167.

Age Determination and Growth of Pacific Ocean Perch (*Sebastes alutus*) in the Northeast Pacific Ocean

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WESTRHEIM, S. J. 1973. Age determination and growth of Pacific ocean perch (*Sebastes alutus*) in the northeast Pacific Ocean. J. Fish. Res. Board Can. 30: 235–247.

Age was determined by means of otoliths of juvenile and adult Pacific ocean perch (*Sebastes alutus*) collected during 1963–69 in the northeast Pacific Ocean, from Oregon to Unalaska Island, Alaska. Consistency of otolith readings between two readers varied inversely with numbers of translucent (or opaque) zones — from 100% agreement for 0-zone otoliths to 23% for 19-zone otoliths. Variability in zone counts between two readers was not great — 85% ± 1 zone for 3- to 24-zone otoliths — and the dispersion was near-normal about zero. Validity of otolith readings was established by demonstrating the annual formation of the translucent zone for juvenile fish, and by detecting the temporal passage of dominant year-classes among juveniles and adults.

Age–length relationship varied inversely with depth off British Columbia, and inversely with latitude (or longitude) in the study region. Off Vancouver Island (48°48′N lat), two partially mixed stocks with near-opposite seasonal availability maxima were delineated, from bathymetric age–length and catch-rate data. Latitudinally, there was a sharp discontinuity in the age–length relationship at Dixon Entrance (54°40′N lat).

Ranges of von Bertalanffy growth parameters were as follows: L_∞, 37.2–48.2; K, 0.078–0.169; and t_0, −0.4 to −1.8. No geographical trend was evident for any parameter. Values of L_∞ were larger for females, while values of K were smaller. Considerable variation in parameter values exists among studies on Pacific ocean perch from the western Gulf of Alaska.

Age-weight relationship was generally sigmoid in shape. Values of W_∞ ranged from 791 to 1686 g. Annual weight increments were maximal at ages 7–11.

WESTRHEIM, S. J. 1973. Age determination and growth of Pacific ocean perch (*Sebastes alutus*) in the northeast Pacific Ocean. J. Fish. Res. Board Can. 30: 235–247.

Nous avons déterminé l'âge, à l'aide des otolithes, de perches de mer du Pacifique (*Sebastes alutus*) jeunes et adultes, récoltées entre 1963 et 1969 dans le nord-est du Pacifique, depuis l'Orégon jusqu'à l'île Unalaska, Alaska. L'uniformité des lectures d'otolithes par deux personnes varie en proportion inverse du nombre de zones transparentes (ou opaques) — d'un accord de 100% sur des otolithes de 0 zone à 23% sur des otolithes de 19 zones. La variabilité du nombre de zones comptées par deux lecteurs n'est pas très grande — 85% ± 1 zone sur des otolithes de 3 à 24 zones — et la dispersion est à peu près normale autour de zéro. Nous avons vérifié la validité des lectures en démontrant la formation d'une zone translucide annuelle chez les jeunes poissons, et en observant l'évolution dans le temps d'une classe-âge dominante chez les jeunes et les adultes.

Au large de la Colombie-Britannique, la relation âge–longueur varie inversement avec la profondeur et, dans toute la région étudiée, elle varie inversement avec la latitude (ou la longitude). A partir de données bathymétriques âge–longueur et taux de capture, nous avons identifié au large de l'île Vancouver (48°48′ lat. N) deux stocks partiellement mélangés, dont la disponibilité saisonnière maximale est presque à l'opposé l'une de l'autre. La relation âge–longueur présente une brusque discontinuité latitudinale à l'entrée Dixon (54°40′ lat N).

La gamme des paramètres de croissance de von Bertalanffy est la suivante: L_∞, 37.2 à 48.2; K, 0.078 à 0.169; et t_0, −0.4 à −1.8. Les valeurs de L_∞ sont plus grandes, et celles de K plus petites, chez les femelles que chez les mâles. Les études sur la perche de mer du secteur ouest du golfe d'Alaska démontrent qu'il existe de grandes variations dans la valeur de ces paramètres.

La courbe âge–poids est ordinairement sigmoïde. Les valeurs de W_∞ varient de 791 à 1686 g. La croissance pondérale annuelle est maximale entre les âges de 7 et 11 ans.

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AGE determination and growth of rockfishes (Scorpaenidae) off the west coast of North America have received little attention. The few studies published have been limited geographically. In California, Wales (1952) studied the blue rockfish (*Sebastes mystinus*), and Phillips (1964) reported on 10 species, not including Pacific ocean perch (*S. alutus*). The first study of Pacific ocean perch age determination and growth (Westrheim 1958) was based on specimens collected from commercial trawl catches taken off the States of Oregon and Washington. The results were subsequently summarized in Alverson and Westrheim (1961). Lyubimova (1964) and Chikuni (1970, 1971) have investigated Pacific ocean perch age and growth in the Gulf of Alaska. In the Bering Sea, similar studies have been reported by Moiseev and Paraketsov (1961), Gritsenko (1963), Paraketsov (1963), Chikuni (1968a, b), and Chikuni and Wakabayashi (1970). All investigators utilized scales for age determination.

In 1962, the Fisheries Research Board of Canada initiated an investigation into the distribution, abundance, and biology of Pacific ocean perch in the northeast Pacific Ocean. One phase of the study involved age determination and growth. This report summarizes the results obtained from collections during 1963–69.

Materials and Methods

STUDY REGION

The study region extended from Cape Blanco, Oregon, to Unalaska Island, Alaska, and was divided into five areas (Fig. 1), consolidated from the 14 areas of Westrheim (1970).

COLLECTION OF MATERIAL

Initially, both scales and otoliths were collected, but the latter were soon selected as the preferred age indicator. Otoliths were readable to an older age than were scales, and were easier to collect and process. Westrheim (1958) and Gritsenko (1963) reported difficulty in detecting scale annuli beyond the 12th. They also noted the high frequency of regenerated scales, particularly among adult specimens. All otoliths were stored in 50% glycerine shortly after collection.

Virtually all specimens were collected aboard the research vessel *G. B. Reed*. During 1963–66, a trawl and echo-sounder survey was conducted on the rockfish stocks in the northeast Pacific Ocean (Westhreim 1970), and otolith samples were collected intermittently throughout the survey area. Sample lots consisted of one or more tubs of Pacific ocean perch randomly selected from the catches. For each fish in the sample lot, fork length (nearest lower centimeter), sex, and gonad condition were recorded, and the left otolith was removed. If this otolith was broken or crystallized, the right otolith was collected. When large numbers of juvenile fish were sampled, a stratified otolith sample was selected, normally from 10 fish per centimeter interval of fork length.

A special search for juvenile Pacific ocean perch off British Columbia was undertaken in 1963 and 1964 that resulted in an extended study during 1963–68 in Smith Sound — an inlet off southeastern Queen Charlotte Sound (Fig. 1). For this work, the conventional groundfish trawl (Westrheim 1970) was replaced with a small shrimp trawl (Greenwood 1959, fig. 3) having 1.5-inch mesh, and a 0.5-inch mesh codend liner.

Beginning in 1967 the otolith sampling procedure was changed. For the standard 6-tub catch sample (West-

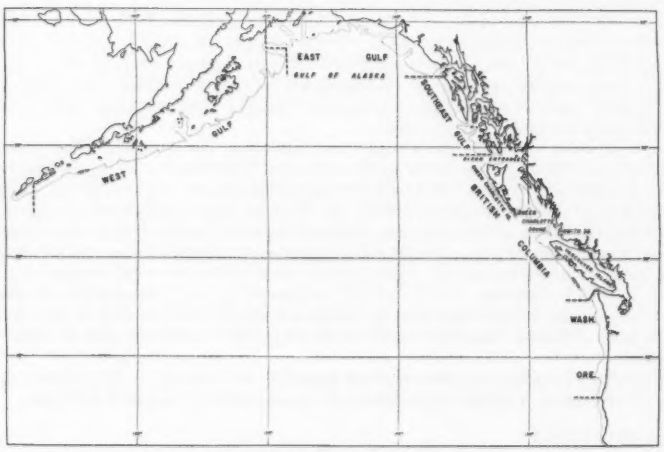

FIG. 1. Chart of the study area.

rheim 1967), otoliths from the fish in the first two and last two tubs were collected by sex in jars, without reference to length. From fish in the middle two tubs, otoliths were collected in vials by sex and length — one vial per sex for each centimeter interval from 23 to 50 cm, and one vial per sex for all otoliths from fish less than 23 cm (normally a minor component of the catch). This innovation was developed to reduce catch-processing time at sea, coincidental with a change in emphasis of our studies from age determination and growth to age composition and stock assessment.

Identification of Juveniles

Identification of juvenile Pacific ocean perch, particularly specimens less than 20 cm long, was not always positively established due to their similar appearance with other juvenile rockfish species. There is no published key for juvenile *Sebastes*. However, occasional tests of our identification were made by hemoglobin protein electrophoresis (Tsuyuki et al. 1968) on juveniles as small as 10 cm, and all results were positive. The following one-species key was devised to assist in identification of juvenile Pacific ocean perch (nomenclature as in Phillips 1957):

A, Spines on top of head: nasal, preocular, supraocular, postocular, tympanic, and parietal. Coronal and nuchal spines absent.

B, Diagonal rows of scales below lateral line: 49–55.

C, Four black, or olive, bandlike blotches on back.

Item A eliminates all *Sebastes* except *alutus*, *crameri* (if nuchals coalesced with parietals), *entomelas*, *ovalis*, and *reedi*.

Item B eliminates all remaining *Sebastes* except *alutus* and *crameri*.

Item C eliminates *crameri*.

Otolith Reading

Otoliths were read on their distal surface under reflected light with a binocular microscope, at 10 magnifications. Summer growth zones appeared as relatively broad, opaque white bands, and winter zones as relatively narrow, translucent blue bands (Fig. 2). One opaque band plus the adjacent, outer translucent band was considered to represent 1 year of life of the fish. Annuli were counted only along the "northeast" or "south" axis ("northwest" for right otoliths). Readability was not consistently good on other axes of the otoliths.

A code was devised to indicate the status of the outer edge of the otolith. For example,

2+ two complete opaque zones, each surrounded by a translucent zone, plus an opaque zone on the outer edge of the "northeast" and/or "south" axis.

3– same as above, except that a translucent zone has formed on the outer edge of either the "northeast" or "south" axis, but not both.

3 three complete opaque zones, with a translucent zone on the outer edge of the otolith on both the "northeast" and "south" axes.

These criteria were normally applicable only to otoliths possessing eight or less opaque zones.

For the 1963–66 collections, each otolith was read independently by two readers (A and B), without reference to length or sex of the fish. If these two readings did not agree (by year-class), the two readers attempted to resolve their disagreement. If they failed, the author rendered a final interpretation.

For the 1967–69 collections, each otolith was read once by reader A or reader B. Periodic comparisons were made between readers to ensure consistent interpretations.

Growth Parameters

Length–weight formulae ($W = aL^b$) used to compute W_∞ and w_ℓ were from Westrheim (1967) and Westrheim and Thomson (1970), and the parameters for metric units (centimeters and grams) are as follows:

		Oreg.–SE Gulf	E Gulf–W Gulf
Males	*a*	0.011348	0.026233
	b	3.06514	2.79344
Females	*a*	0.0078571	0.035508
	b	3.16734	2.70836
Males + Females	*a*	0.0089550	0.032026
	b	3.13325	2.72733

Computations of the von Bertalanffy growth parameters L_∞, K, and t_o were performed by computer using the method developed by Allen (1966). Age-group mean lengths were used in lieu of individual lengths at age.

Age Determination

Consistency of Readings

Percentage agreement of independent readings by two readers varied inversely with numbers of translucent (or opaque) zones, and did not differ appreciably by sex or area. For 5496, 0–9-zone otoliths, agreement declined from 100% for 0-zone otoliths to 53% for 9-zone otoliths (Table 1). For 0–4-zone otoliths, agreement was usually 90% or more. For 4698, 10–20-zone otoliths, agreements declined from 54% for 10-zone otoliths to 23% for 19-zone otoliths (Table 2).

Variability of zone-counts among readers was not great. For 900 randomly selected, 3–24-zone otoliths, 85% of reader B readings deviated from those of reader A by no more than ±1 zone (Table 3), and 94% by no more than ±2 zones. Notably, the distribution of deviations was near-normal about zero. Dispersion about zero was greater for 16–24-zone otoliths (39–59% ± 1 zone; 63–82% ± 2

zones) than for 3–15-zone otoliths (69–100% ± 1 zone; 83–100% ± 2 zones).

Validity of Readings

Two kinds of indirect evidence (Van Oosten 1929) were available to assess the validity of the otolith readings. We have demonstrated that the translucent zone is formed annually on otoliths of juvenile fish, and we detected the progression of dominant year-classes among juvenile and adult fish. There was no direct evidence available.

Annual zone formation — In March 1963, a small stock of juvenile Pacific ocean perch was discovered in Smith Sound; subsequently sampled periodically throughout 1964; and sampled annually in February or March during 1964–68. Translucent zones began forming as early as October on some otoliths, and virtually all otoliths exhibited complete or nearly complete translucent zones by the following March. Substantiating data for the 1956 and 1962 year-classes have been selected as an example (Table 4). For the 1956 year-class, the 1963 translucent zone was completely formed on all 30 otoliths examined

Table 1. Consistency (percent agreement) of readings of 0–9-zone otoliths of Pacific ocean perch (*Sebastes alutus*) by area of collection, 1963–68.

No. of zones	Smith Sound						Other B.C. areas		Gulf of Alaska		Total	
	Males		Females		Unknown		M+F		M+F		M+F	
	T	%	T	%	T	%	T	%	T	%	T	%
0	–	–	–	–	–	–	49	100	–	–	49	100
1	–	–	–	–	1	100	348	97	–	–	349	97
2	74	100	92	98	241	99+	495	86	192	96	1094	93
3	65	98	97	95	85	99	505	90	127	88	879	92
4	169	98	168	95	35	89	698	93	352	80	1422	90
5	32	78	42	81	17	94	149	76	165	64	405	72
6	14	64	20	85	7	57	129	67	137	47	307	59
7	83	87	83	82	40	80	69	39	136	54	411	66
8	148	70	172	66	17	65	33	61	59	42	429	63
9	38	55	42	55	6	67	14	36	51	53	151	53
T	623		716		449		2489		1219		5496	

Table 2. Consistency (percent agreement) of readings of 10–20-zone otoliths of Pacific ocean perch, by area o collection, 1963–66.

No. of zones	Oreg.–Wash.				B.C.				Gulf of Alaska				Total	
	Males		Females		Males		Females		Males		Females		M+F	
	T	%	T	%	T	%	T	%	T	%	T	%	T	%
10	24	50	16	50	62	56	46	54	183	52	197	57	528	54
11	49	41	34	47	92	39	100	46	202	47	220	53	697	47
12	30	43	53	43	136	57	115	54	197	45	179	41	710	47
13	57	21	50	40	170	44	117	45	113	36	146	38	653	39
14	42	40	40	35	160	41	153	50	62	39	80	35	537	42
15	44	36	20	20	124	40	128	42	53	19	67	29	436	35
16	33	27	15	33	79	24	80	30	31	19	45	24	283	26
17	14	36	16	13	78	22	77	27	19	20	35	26	239	24
18	27	33	23	17	72	31	73	22	12	8	29	27	236	25
19	20	15	26	19	49	24	69	25	6	17	21	28	191	23
20	3	67	19	47	53	13	84	29	10	30	19	16	188	26
Total	343		312		1075		1042		888		1038		4698	

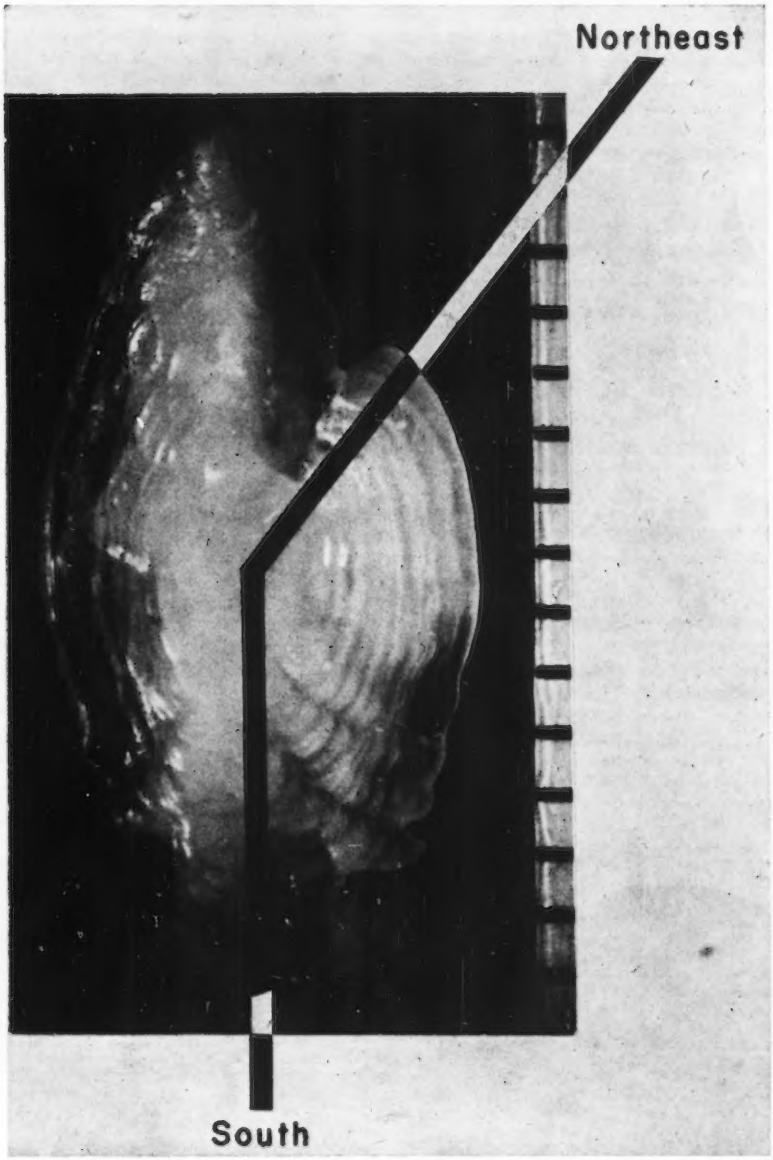

FIG. 2. Photograph of the left otolith from a 36-cm female Pacific ocean perch (*Sebastes alutus*) (age 15 years), with northeast and south axes superimposed. (*Scale in millimeters*).

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TABLE 3. Deviations of otolith readings by reader B from those by reader A, by zone group, for selected *G. B. Reed* samples, 1964–65.

Deviation (A–B)	Number of zones																	Total
	3–5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21–24	
+6	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	1	2
+5	–	–	–	–	–	–	–	–	–	–	1	–	–	–	–	0	1	2
+4	–	–	–	–	–	–	–	–	–	2	0	1	–	–	1	0	2	6
+3	–	–	–	–	–	–	1	2	1	3	0	2	1	3	0	0	0	13
+2	–	2	–	2	4	1	4	5	4	2	2	0	1	1	0	3	3	34
+1	8	2	5	15	13	16	23	16	19	10	10	6	3	1	0	3	0	150
0	154	21	20	31	54	39	42	38	22	17	11	5	4	7	3	5	3	476
–1	4	4	9	6	12	15	24	19	16	6	6	2	3	1	3	2	4	136
–2	–	1	–	1	2	4	12	6	5	5	1	1	3	0	2	1	4	48
–3	–	–	–	–	–	0	1	4	1	1	1	1	3	0	2	0	–	14
–4	–	–	–	–	–	0	–	2	0	1	0	3	1	0	1	1	–	9
–5	–	–	–	–	–	1	–	–	0	0	1	1	0	2	–	0	–	5
–6	–	–	–	–	–	–	–	–	0	1	–	–	1	1	–	1	–	4
–9	–	–	–	–	–	–	–	–	1	–	–	–	–	–	–	–	–	1
T	166	30	34	55	85	76	107	92	69	48	33	22	20	16	12	17	18	900
%±1	100	90	100	95	93	92	83	79	83	69	82	59	50	56	50	59	39	85
%±2	100	100	100	100	100	99	98	91	96	83	91	64	70	63	67	82	78	94

TABLE 4. Zone formation on juvenile Pacific ocean perch otoliths, 1956 and 1962 year-classes, Smith Sound, 1963–68. (Numbers of otoliths by age code.)

	1963	1964					1965	1966	1967	1968
Age code	Mar.	Mar.	May	July	Oct.	Dec.	Feb.	Mar.	Mar.	Feb.
					1956					
7–	18	–	–	–	–	–	–	–	–	–
7	65	–	–	–	–	–	–	–	–	–
7+	2[a]	–	–	–	–	–	–	–	–	–
8–	–	56	–	–	–	–	–	–	–	–
8	–	76	30	81	27	–	–	–	–	–
8+	–	–	–	2	5	7	–	–	–	–
9–	–	–	–	–	12	10	5	–	–	–
9	–	–	–	–	–	10	24	–	–	–
9+	–	–	–	–	–	–	–	–	–	–
10–	–	–	–	–	–	–	–	1	–	–
10	–	–	–	–	–	–	–	12	–	–
					1962					
2–	–	3	–	–	–	–	–	–	–	–
2	–	210	–	–	–	–	–	–	–	–
2+	–	4[a]	15	21	55	9[b]	–	–	–	–
3–	–	–	–	–	37	36	12	–	–	–
3	–	–	–	–	–	14	93	–	–	–
3+	–	–	–	–	–	–	–	9[b]	–	–
4–	–	–	–	–	–	–	–	70	–	–
4	–	–	–	–	–	–	–	217	–	–
4+	–	–	–	–	–	–	–	6[a]	22[b]	–
5–	–	–	–	–	–	–	–	–	142	–
5	–	–	–	–	–	–	–	–	73	–
5+	–	–	–	–	–	–	–	–	–	22[b]
6–	–	–	–	–	–	–	–	–	–	41
6	–	–	–	–	–	–	–	–	–	7

[a]New growth.
[b]Old growth.

in May 1964. The 1964 translucent zone first appeared on otoliths collected in October (12 of 44 examined). In the February 1965 sample, all 29 otoliths examined exhibited a complete or nearly complete 1964 translucent zone. For the 1962 year-class, the 1963 translucent zone was evident on one or both axes of all 217 otoliths collected in March 1964. The 1964 zone first appeared on otoliths collected in October (37 of 92 examined). In February 1965, all 105 otoliths collected exhibited a complete or nearly complete 1964 translucent zone.

Dominant year-classes — During 1963–68 in Smith Sound, the 1956, 1962, and 1964 year-classes were relatively dominant, particularly the 1962 year-class which was first noted in 1964 as 2-year fish (modal size, 14 cm) and was still prominent in 1968 as 6-year fish (modal size, 22 cm) (Fig. 3). On the nearby commercial fishing grounds in Queen Charlotte Sound, the 1952 year-class was relatively prominent

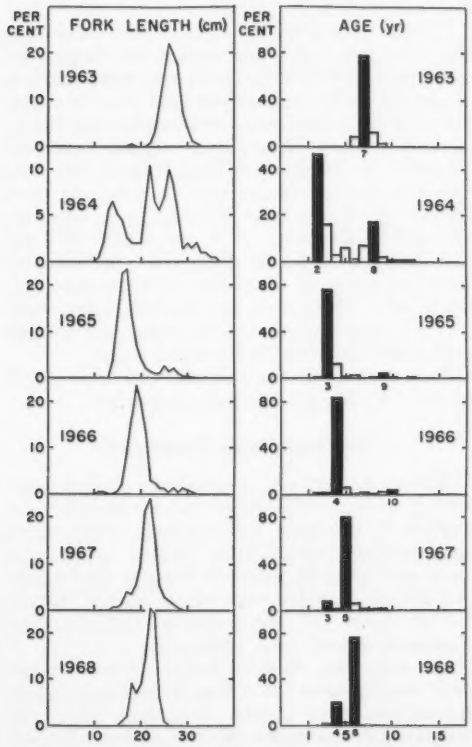

FIG. 3. Size and age composition of juvenile Pacific ocean perch in Smith Sound, February–March 1963–68.

in the British Columbia trawl landings and *G. B. Reed* catches during 1963–67 (Fig. 4). Its apparent loss of dominance as 17-year fish in 1969 has been attributed in part to age determination difficulties. Notable in the *G. B. Reed* on-bottom trawl catches of 1967 and 1969 are the 1962 and 1964 year-classes.

During 1963–65 in the SE Gulf–W Gulf region, the 1953 year-class was prominent in the *G. B. Reed* catches (Westrheim 1970, fig. 10). During 1965–67 in the SE Gulf area, the 1961 year-class was prominent (Fig. 5).

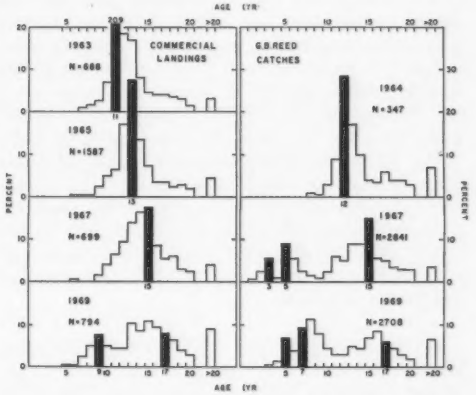

FIG. 4. Age composition of Pacific ocean perch in commercial landings and *G. B. Reed* catches from Queen Charlotte Sound, 1963–69.

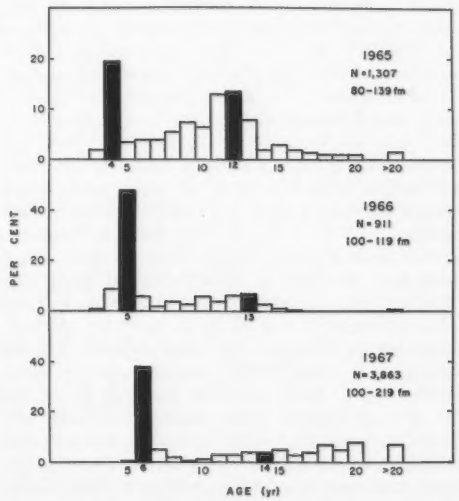

FIG. 5. Age composition of Pacific ocean perch in *G. B. Reed* catches from the SE Gulf area, 1965–67.

Age–Length Relationship

Pacific ocean perch age–length relationships proved to be complex for both juvenile and adult fish. For juveniles, individual age-group mean lengths varied among subareas off British Columbia, and among areas within the study region. Within the British Columbia area, mean lengths per age-group were higher off southwest Vancouver Island, and lower in Smith Sound, than in Queen Charlotte Sound. For instance, during February–March, mean lengths of 3-year fish were 17.1 cm in Smith Sound, 17.8 cm in Queen Charlotte Sound, and 19.2 cm off southwest Vancouver Island (Table 5). Among areas, mean lengths per age-group generally declined northward and westward. In the July–September samples, mean lengths of 4-year fish were 20.2 cm off southwest Vancouver Island; 17.7 cm in SE Gulf; 17.6 cm in E Gulf; and 16.8 cm in W Gulf.

For adult fish, bathymetric and latitudinal variations in the age-length relationship were noted. Mean lengths of each age-group varied inversely with depth and latitude (or longitude). The bathymetric variation was noted only within the British Columbia area. Elsewhere, otolith sampling by depth was insufficient.

Bathymetric variation — Off southwest Vancouver Island, sufficient data were collected during September 1965–66, February 1969, and June 1968 to demonstrate a bathymetric variation in the age–length relationship. For both sexes, age-group mean lengths were relatively large in shallower water, declined sharply at intermediate depths, and were relatively small in deeper water. Hence, it appeared that this stock consisted of two types of fish, shallow and deep, which were mixed at intermediate depths.

Using age-group 14 as an example, for shallow-water males (Fig. 6) mean-length ranges were 38.4–40.3 cm (80–139 fath) in September; 37.8–38.5 cm (140–179 fath) in February, and 37.1 cm (140–159 fath) in June. For deepwater males, mean-length ranges were 35.5–36.8 cm (140–199 fath) in September; 34.7–35.1 cm (200–279 fath) in February; and 34.6–34.8 cm (180–239 fath) in June. Catch rates were maximal at 100–139 fath in September, 200–239 fath in February, and 160–219 fath in June. The substantially lower catch rates for shallow-water fish in February and June, together with the reduction in mean length, suggest that the larger shallow-water males were not available to capture by our on-bottom trawl during February and June. For deepwater males, age-group mean lengths remained remarkably uniform among months, but catch rates were maximal in February. Presumably, the deepwater males form denser aggregations during February. Deepwater males also exhibited seasonal bathymetric migration. Their upper limit was at least 140–159 fath in September; 200–219 fath in February; and 180–199 fath in June.

For females, a similar situation was evident (Fig. 6). For age-group 14, mean-length ranges for shallow-water fish were 40.5–41.5 cm (80–139 fath) in September; 37.8 cm (160–179 fath) in February; and 38.6 cm (140–159 fath) in June. For deepwater females, the corresponding ranges were 35.9–36.6 cm (140–219 fath) in September; 35.6–36.8 cm (180–279 fath) in February; and 35.8–36.3 cm (160–239 fath) in June. Catch rates were maximal at 100–119 fath in September; 200–239 fath in February; and 160–219 fath in June. As with males, the larger shallow-water females were not availabie during February and June, and deepwater females formed denser aggregations in February. Similarly, deepwater females also exhibited a seasonal bathymetric migration. Their upper limit was at least 140–159 fath in September; 180–199 fath in February; and 160–179 fath in June.

Geographical variation — For shallow-water Pacific ocean perch collected during July–September, mean lengths of individual adult age-groups generally decreased northward and westward (Fig. 7). Pacific ocean perch in the Oregon–Washington and British Columbia areas differed only slightly from one another, but substantially from those in the areas north of Dixon Entrance. For example, the mean lengths of 14-year males by area were 38.6 cm (Oregon–Washington), 37.7 cm (B.C.), 33.7 cm (SE Gulf), 34.7 cm (E Gulf), and 33.9 cm (W Gulf). Comparable values for 14-year females were 38.5, 38.7, 34.7, 35.6, and 34.5 cm, respectively. Dixon Entrance appears to be a boundary between two growth types of Pacific ocean perch.

The anomalous mean lengths for ages 14–20 in the E Gulf area were not explainable.

Von Bertalanffy Parameters

Before computing von Bertalanffy growth parameters, some consolidations and adjustments were applied to the data. For interarea comparisons, only summer collections in shallow water were used, and the data from the Oregon–Washington and British Columbia areas were combined. Within the B.C. area, all the deepwater collections off Vancouver Island were combined.

For each area, all mean lengths of juvenile and adult Pacific ocean perch were plotted against age. Anomalous mean lengths, apparently biased by emigration or incomplete recruitment, were eliminated by inspection.

For the growth analysis of both shallow and deep Pacific ocean perch in the Oregon–British Columbia

TABLE 5. Age-group mean lengths ($\bar{L}$ = cm), and numbers (N) of otoliths read, for juvenile Pacific ocean perch (sexes combined), by area and season, 1963–68.

Age-group (*years*)	Smith Sound				Queen Charlotte Sound				SW Vancouver Island				SE Gulf		E Gulf		W Gulf			
	Oct.		Feb.–Mar.		July		Feb.–Mar.		Sept.		Feb.–Mar.		Sept.		July–Sept.		July–Aug.[a]		Feb.[b]	
	$\bar{L}$	N	$\bar{L}$	N	$\bar{L}$	N	$\bar{L}$	N	$\bar{L}$	N	$\bar{L}$	N	$\bar{L}$	N	$\bar{L}$	N	$\bar{L}$	N	$\bar{L}$	N
1	–	0	–	0	–	0	8.3	20	6.6	99	10.0	13	–	0	–	0	–	0	–	0
2	11.5	1	13.2	372	10.4	254	13.9	261	12.4	158	15.5	60	–	0	–	0	–	0	14.5	1
3	16.4	222	17.1	527	15.4	2961	17.8	112	16.8	3	19.2	270	14.5	2	14.9	474	13.5	1	17.3	71
4	19.9	32	20.0	86	19.4	668	20.0	2	20.2	66	21.8	251	17.7	32	17.6	26	16.8	14	20.1	104
5	23.0	4	21.8	49	22.1	488	22.0	4	22.8	31	24.0	55	22.4	550	20.7	20	18.4	315	22.5	37
6	23.5	26	24.7	31	23.5	46	–	0	24.7	6	25.6	14	24.9	66	22.1	8	21.7	1350	24.0	97
7	26.2	9	25.8	143	25.6	72	24.5	1	26.5	1	27.2	7	27.0	40	23.2	12	22.7	126	25.1	55
8	26.6	72	26.5	155	27.2	29	–	0	26.5	1	28.5	5	28.6	9	23.8	75	23.8	63	26.9	37
9	27.0	57	27.7	48	28.2	16	–	0	–	0	29.5	1	29.4	13	24.4	8	24.8	52	26.1	5
10	29.0	8	–	0	30.2	8	–	0	–	0	–	0	30.6	34	25.5	1	25.6	15	–	0
Total		431		1411		4542		400		365		676		746		624		2223		407

[a]Principally from off the Shumagin Islands (150°W long).
[b]Off Kodiak Island (152°W long).

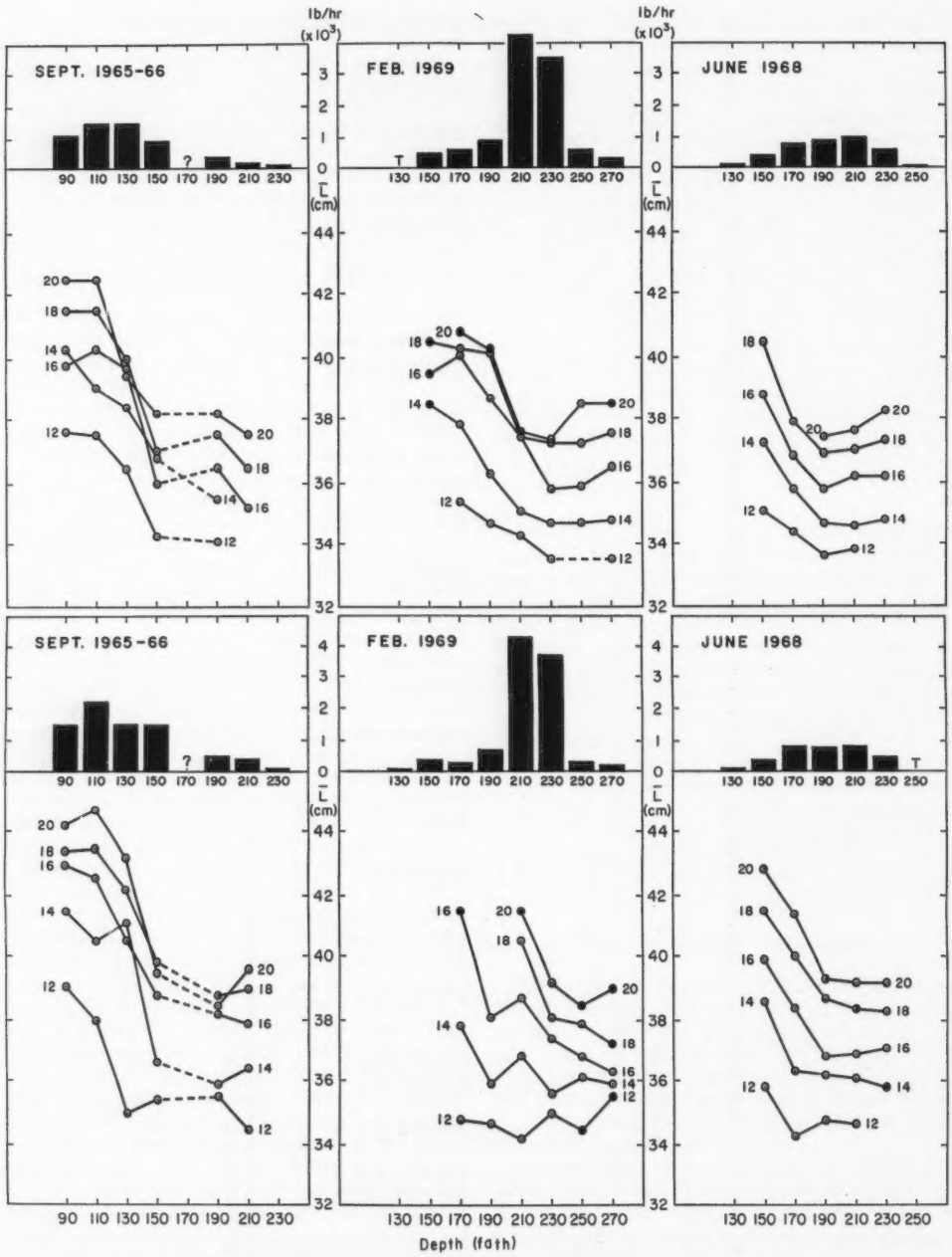

FIG. 6. Mean lengths (*cm*) of selected age-groups, and *G. B. Reed* catch rates (no./hr), by 20-fath depth interval, for male (*upper*) and female (*lower*) Pacific ocean perch off SW Vancouver Island in September 1965–66, February 1969, and June 1968. (Solid dots indicate means based on less than 5 fish.)

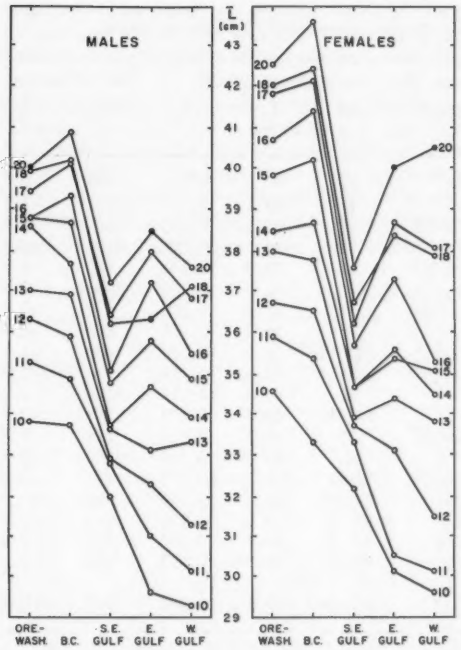

FIG. 7. Mean lengths (*cm*) of selected age-groups collected in shallow water, by area, by sex, summer 1963–69. (Solid dots indicate less than five otoliths.)

region, the shallow-water juvenile growth data collected in September off Vancouver Island were used. Juvenile Pacific ocean perch were not caught in any deepwater area. This procedure was adopted after a brief investigation suggested that the early growth histories of shallow- and deep-types were quite similar. Radii of the first four annuli were measured on 34 otoliths from shallow-type Pacific ocean perch (ages 3–17), and 22 otoliths from deep-type fish (ages 10–23). Deep-type otoliths possessed larger radii ($\bar{x}$ = 10.2 – 27.7) than shallow-type otoliths ($\bar{x}$ = 9.6 – 26.6), but only the mean radii for the first and third annuli were significantly different by depth (P = .05).

In this study, L_∞ values ranged from 37.2 to 48.2 cm (Table 6), and were larger for females than for males in all areas. No geographical trend in values was evident.

K values ranged from 0.078 to 0.169 and were larger for males than for females in all areas. No geographical trend in values was evident.

Values for t_o ranged from –0.4 to –1.8, and were usually larger for females than for males. All areas yielded reasonable values (–0.4 to –0.8), except E Gulf (–1.8), where L_∞ values were relatively high (45.8 and 47.4 cm), and K values low (0.083 and 0.078).

In the Oregon–Washington area, Alverson and Westrheim (1961) reported somewhat lower values of L_∞ (males = 40.3 cm; females = 43.2 cm), and higher values of K (males = 0.20; females = 0.18), than those for this study in the Oregon–British Columbia area (L_∞ = 43.3 and 48.2; K = 0.135 and 0.106).

In the W Gulf area, Lyubimova's (1964) calculated age–length relationship yielded values of L_∞ (42.2 cm) and K (0.156) higher than those of this study (L_∞ = 40.8 cm; K = 0.113). Chikuni (1971) computed two sets of parameters to describe the age–length relationship, and hence they are difficult to compare with those of this study.

In the Bering Sea, the parameters computed from the calculated age–length relationships of Moiseev and Paraketsov (1961) and Gritsenko (1963) differed substantially from those of Lyubimova or this study. L_∞ values (49.9 and 52.1 cm) were higher, and K values (0.094 and 0.086) were lower.

Calculated mean lengths per age-group generally declined northward and westward for both sexes (Fig. 8, Table 7). Females had a larger mean length per age-group, but only slightly so north of British Columbia.

Within the Oregon–British Columbia region, the calculated mean lengths for shallow-water Pacific ocean perch agreed reasonably well with the corrected values of Alverson and Westrheim (1961) for the Oregon–Washington area (Table 7).

In the W Gulf area, the age–length relationship (sexes combined) from this study differed from those of Lyubimova (1964) and Chikuni (1971), both of whom utilized back calculations from scale measurements. Calculated mean lengths per age-group were smaller in this study than in those of Lyubimova and Chikuni (Table 7). For example, mean lengths for age-groups 3 and 11 were 15.5 and 34.1 cm (Lyubimova); and 17.9 and 31.6 cm (Chikuni); compared with 13.5 and 30.0 cm for this study. Lyubimova and Chikuni noted an increase in the growth rate of juvenile fish — at age 3 and 4.6, respectively — and attributed this to the juvenile fish shifting from pelagic to benthic existence. An equally plausible explanation is the differential emigration of juvenile Pacific ocean perch to the deeper waters occupied by the adults. However, until more detailed studies are undertaken, the problem will remain unsolved.

Age–Weight Relationship

In the Oregon–British Columbia region, the age–weight relationship was generally sigmoid in shape

TABLE 6. Von Bertalanffy growth parameters for Pacific ocean perch, by area or region.

Area/Region	Source	Relative depth	Age-groups[a]	Sex	L_∞ (*cm*)	K	W_∞	t_o
Oreg.–Wash.	Alverson and Westrheim (1961)	Shallow	1–14[b]	M	40.3	0.20	934	–0.7
				F	43.2	0.18	1152	–1.1
Oreg.–B.C.	This study	Shallow	1–6[c];11–21[d]	M	43.3	0.135	1176	–0.4
				F	48.2	0.106	1686	–0.7
Vancouver Is.	This study	Deep	1–6[c];14–21[e]	M	38.9	0.156	848	–0.4
				F	41.6	0.134	1056	–0.5
SE Gulf	This study	Shallow	3–10[f];11–21	M	37.2	0.169	739	–0.4
				F	38.5	0.153	826	–0.7
			3–8[f];11–21[d]	M+F	38.6	0.141	838	–1.1
E Gulf	This study	Shallow	3–7[f];11–16[d]	M	45.8	0.083	1144	–1.8
				F	47.4	0.078	1229	–1.8
				M+F	48.3	0.074	1254	–2.0
W Gulf	This study	Shallow	3–7[f];12–17[d]	M	40.5	0.113	811	–0.7
				F	41.7	0.107	868	–0.8
			4–7[f];12–17[d]	M+F	40.8	0.113	791	–0.6
	Lyubimova (1964)	?	1–19	M+F	42.2	0.156	903	+0.1
	Chikuni (1971)[g]	?	<4.6	M+F	29.0	0.341	316	+0.2
			>4.6	M+F	41.1	0.104	887	+3.0
Bering Sea	Moiseev and Paraketsov (1961)	?	1–21[b]	M+F	49.9	0.094	1432	–0.5
	Gritsenko (1963)	?	1–17[b]	M+F	52.1	0.086	1612	–0.4

[a]Age-group 1 = ages 0.1–1.0; age-group 2 = ages 1.1–2.0; etc.
[b]Back-calculations to time of annulus formation (ages 1.0, 2.0, etc.)
[c]Table 5; SW Vancouver Island, September.
[d,e]Supporting data available from author.
[f]Table 5.
[g]Two formulae used to describe growth.

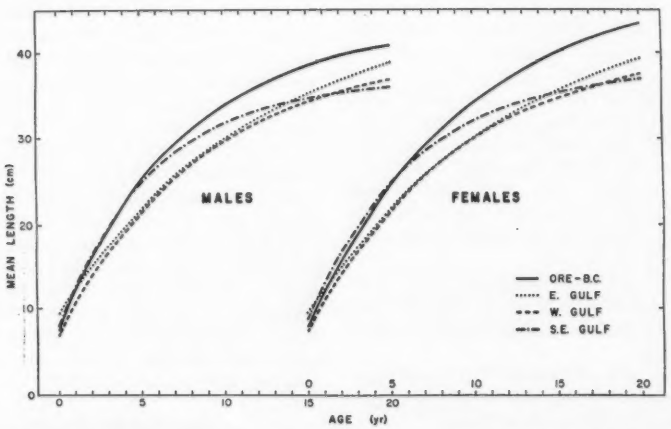

FIG. 8. Calculated age–length relationships, by area, for male and female Pacific ocean perch collected in shallow water, summer season. Calculations made from von Bertalanffy growth parameter in Table 6.

(Fig. 9). Calculated asymptotic weights (W_∞) ranged from 848 to 1686 g (Table 6). Shallow-water Pacific ocean perch were heavier per age-group than deepwater fish, and females were heavier per age-group than males. Annual weight increments increased quickly, to a peak of 58–80 g at age 7–11, then declined steadily. Shallow-water fish had larger annual weight increments than those from deep water, and maxima were reached at an older age, 8 and 11 vs. 7 and 9. Female Pacific ocean perch had larger annual weight increments than males, and maxima were reached at an older age, 11 and 9 vs. 8 and 7.

The age–weight relationship reported by Alverson and Westrheim (1961) for the Oregon–Washington area was similar in shape and magnitude. W_∞ was 934 g for males and 1152 g for females. Ages at maximum weight increment were 6 for males and 7 for females.

North of British Columbia, the age–weight relationships of Pacific ocean perch were similar to those in the Oregon–British Columbia region (Fig. 10).

TABLE 7. Calculated mean lengths (*centimeters*) for selected age-groups of Pacific ocean perch.

Age-group (*years*)	Oreg.–Wash.[a]		Oreg.–B.C.[b]		SE Gulf[b]	E Gulf[b]	W Gulf			Bering Sea	
	M	F	M	F	M+F	M+F	M+F[b]	M+F[c]	M+F[d]	M+F[e]	M+F[f]
1	–	–	7.4	7.8	9.9	9.6	6.6	5.2	7.0	6.9	6.4
3	11.9	13.3	15.9	15.5	16.9	14.9	13.5	15.5	17.9	13.8	13.1
5	21.5	22.1	22.4	21.8	22.3	19.5	19.0	22.8	23.3	20.0	19.1
7	27.9	28.3	27.3	26.8	26.3	23.5	23.4	27.7	26.6	25.2	24.7
9	32.1	32.7	31.1	30.9	29.3	26.9	26.9	31.1	29.4	29.6	29.0
11	34.8	35.8	34.0	34.2	31.6	29.8	30.0	34.1	31.6	32.7	32.8
13	36.7	38.0	36.2	36.9	33.3	32.4	32.0	36.7	33.4	36.2	35.4
15	37.9	39.5	37.9	39.1	34.6	34.6	33.8	–	34.8	38.8	38.0
17	38.7	40.6	39.1	40.8	35.6	36.5	35.2	–	–	40.0	40.7
19	39.2	41.4	40.1	42.2	36.3	38.1	36.3	–	–	41.5	–
21	39.6	41.9	40.9	43.4	36.9	39.5	37.2	–	–	–	–

[a]Alverson and Westrheim (1961).
[b]This study — shallow water.
[c]Lyubimova (1964). Not reported by sex.
[d]Chikuni (1971). Not reported by sex.
[e]Moiseev and Paraketsov (1961). Not reported by sex.
[f]Gritsenko (1963). Not reported by sex.

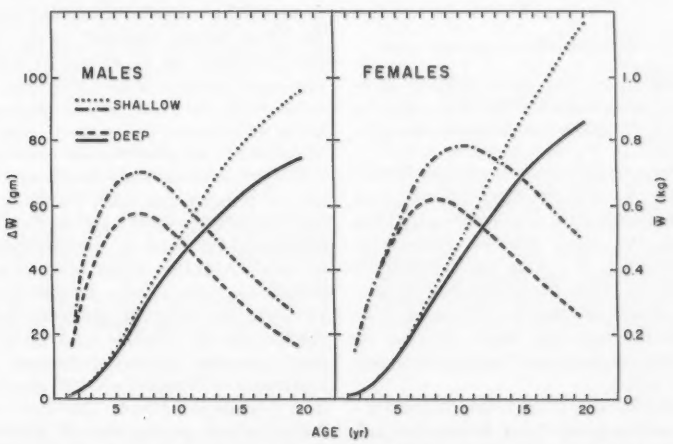

FIG. 9. Calculated age–weight relationship for male and female Pacific ocean perch from shallow and deep water in the Oregon–British Columbia region.

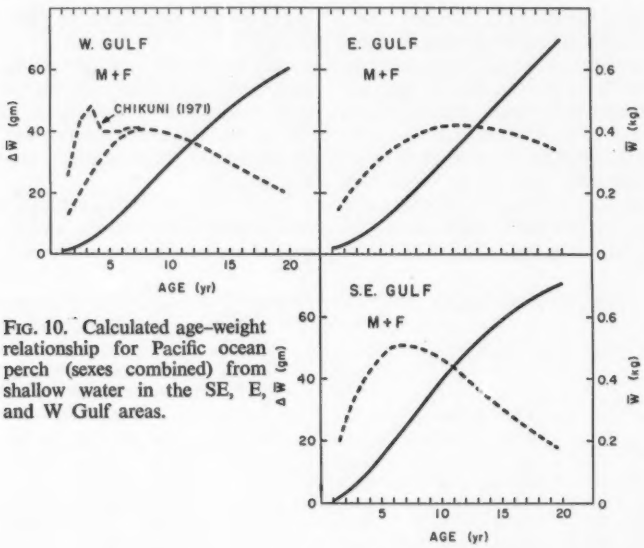

FIG. 10. Calculated age–weight relationship for Pacific ocean perch (sexes combined) from shallow water in the SE, E, and W Gulf areas.

Calculated asymptotic weights (W_∞), for sexes combined, were 838 g in SE Gulf, 1254 g in E Gulf, and 791 g in W Gulf (Table 6). Ages at maximum annual weight increment were 7–8, 12, and 9, respectively, for the same areas. Growth parameters for the E Gulf area were all atypical, compared with those of other areas, and hence are suspect.

Chikuni's (1971, table 6) values for weight increment in the W Gulf area have been plotted in Fig. 10 for comparison. His curve has peaks at ages 4 (47.5 g) and 8 (41.1 g).

Discussion

Growth analysis of Pacific ocean perch is complicated by age determination difficulties, and by bathymetric and geographic variations in the age–length relationship.

Age determination difficulties were noted in this study, particularly for otoliths with 16 or more annuli. Similar problems were noted for scales with 13 or more annuli by other workers (Westrheim 1958; Gritsenko 1963). An apparent example of these difficulties is the disparity in age–length relationship among studies in the W Gulf area. Unfortunately, little evidence has been reported to establish the validity of age determinations obtained from otoliths or scales.

Bathymetric variations in the age–length relationship for Pacific ocean perch were substantial off south-west Vancouver Island, and may well be so elsewhere. Apparently, two partially mixed stocks with near-opposite seasonal availability maxima exist off West Vancouver Island. The apparent shift to deeper water in winter by Pacific ocean perch, as noted by Alverson (1960) and Westrheim (1970) now appears to be a more complicated phenomenon. Most of the fast-growth Pacific ocean perch which were congregated in shallower water during summer either emigrated or dispersed into midwater in winter. Westrheim (1970) noted the substantial decline in catch rate of larger fish from summer to winter. In contrast, the slow-growth Pacific ocean perch from deeper water congregated during winter and dispersed during summer; hence, the bathymetric shift in catch rate by season. It is self-evident that growth analyses could be adversely affected unless adjustments are made for this bathymetric variation in the age–length relationship. Those based on back calculations are particularly vulnerable.

Geographic variations in the age–length relationship of juvenile and adult Pacific ocean perch were also significant. Within the British Columbia area, substantial variation in the age–length relationship of juvenile Pacific ocean perch was noted among subareas — one more complication in growth analysis. The interarea variation in the age–length relationship of juvenile and adult Pacific ocean perch provided additional evidence to support the hypothesis of Westrheim (1970) that Dixon Entrance is a boundary between the B.C.- and Gulf-type Pacific ocean perch, and of Fadeev (1968) that Pacific ocean perch in the Gulf of Alaska are subdivided into individual, nonmigrating stocks.

Acknowledgments

Many individuals contributed to this study, particularly with assistance at sea in collecting and processing otoliths. Messrs D. Davenport and W. R. Harling provided exemplary assistance at sea, and perservered ashore in the unenviable task of interpreting the otoliths. Mr J. A. Thomson provided the necessary support during the computer-processing phase of the study. Drs K. S. Ketchen and W. E. Ricker reviewed the manuscript and offered a number of improvements.

ALLEN, K. R. 1966. A method of fitting growth curves of the von Bertalanffy type to observed data. J. Fish. Res. Board Can. 23: 163–179.

ALVERSON, D. L. 1960. A study of annual and seasonal bathymetric catch patterns for commercially important groundfishes of the Pacific northwest coast of North America. Pac. Mar. Fish. Comm. Bull. 4: 66 p.

ALVERSON, D. L., AND S. J. WESTRHEIM. 1961. A review of the taxonomy and biology of the Pacific ocean perch and its fishery. Rapp. Proces-Verbaux Reunions Cons. Perma. Int. Explor. Mer 150: 12–27.

CHIKUNI, S. 1968a. On the scale characters of the Pacific ocean perch in the Bering Sea. I. Some scale characters and their variations by body regions. Bull. Jap. Soc. Sci. Fish. 34: 681–686.

1968b. On the scale characters of the Pacific ocean perch in the Bering Sea. II. Formation of the resting zone on scale, its time and periodicity. Bull. Jap. Soc. Sci. Fish. 34: 770–774.

1970. On the scale characters of the Pacific ocean perch in the Northeast Pacific Ocean. I. Some scale characters, their variation by body region and formation of the resting zone. Bull. Far Seas Fish. Res. Lab. (Shimizu) 3: 187–204.

1971. On the age and size relationship of the Pacific ocean perch in the northeastern Pacific. Bull. Far Seas Fish. Res. Lab. (Shimizu) 4: 27–49.

CHIKUNI, S., AND K. WAKABAYASHI. 1970. On the scale characters of the Pacific ocean perch in the Bering Sea. III. Objectivity and accuracy of age determination by scale reading. Bull. Far Seas Fish. Res. Lab. (Shimizu) 3: 205–214.

FADEEV, N. S. 1968. Migrations of Pacific ocean perch. Proc. Pac. Sci. Res. Inst. Fish. Oceanogr. 65: 170–177. (Transl. from Russian by Fish. Res. Board Can. Transl. Ser. No. 1447, 1971)

GREENWOOD, M. R. 1959. Shrimp exploration in central Alaska waters by M/V *JOHN N. COBB*, July–August 1958. Commer. Fish. Rev. 21(7): 1–13.

GRITSENKO, O. F. 1963. Age and growth rate of Pacific rockfish of the Bering Sea, p. 328–331. *In* Soviet fisheries investigations in the northeast Pacific. Part I. (Transl. from Russian for Clearinghouse Fed. Sci. Tech. Inform., Springfield, Va., TT67–51203.)

HARLING, W. R., M. S. SMITH, D. DAVENPORT, AND D. M. BIANCHIN. 1969. Preliminary report on maturity, spawning season, and larvae identification of rockfishes (*Sebastodes*) collected in waters off British Columbia during February 1969. Fish. Res. Board Can. MS Rep. 1055: 11 p.

LYUBIMOVA, T. G. 1964. Biological characteristics of the school of Pacific rockfish (*Sebastodes alutus*) in the Gulf of Alaska, p. 208–218. *In* Soviet fisheries investigations in the northeast Pacific. Part III. (Transl. from Russian for Clearinghouse Fed. Sci. Tech. Inform. Springfield, Va., TT67–51205.)

MOISEEV, P. A., AND I. A. PARAKETSOV. 1961. Information on the ecology of rockfishes (family Scorpaenidae) of the northern part of the Pacific Ocean. Vop. Ikhtiol. 1(18): 39–45. (Transl. from Russian by Fish. Res. Board Can. Transl. Ser. No. 358, 1962)

PARAKETSOV, I. A. 1963. On the biology of *Sebastodes alutus* of the Bering Sea, p. 319–327. *In* Soviet fisheries investigations in the northeast Pacific. Part. I. (Transl. from Russian for Clearinghouse Fed. Sci. Tech. Inform. Springfield, Va., TT67–51203.)

PHILLIPS, J. B. 1957. A review of the rockfishes of California (family Scorpaenidae). Calif. Dep. Fish Game Fish Bull. 104: 158 p.

1964. Life history studies on ten species of rockfish (Genus *Sebastodes*). Calif. Dep. Fish Game Fish Bull. 126: 70 p.

TSUYUKI, H., E. ROBERTS, R. H. LOWES, W. HADAWAY, AND S. J. WESTRHEIM. 1968. Contribution of protein electrophoresis to rockfish (Scorpaenidae) systematics. J. Fish. Res. Board Can. 25: 2477–2501.

VAN OOSTEN, J. 1929. Life history of the lake herring (*Leucicthys artedi* Le Seur) of Lake Huron as revealed by its scales, with a critique of the scale method. U.S. Bur. Fish. Bull. 44: 265–428.

WALES, J. H. 1952. Life history of the blue rockfish *Sebastodes mystinus*. Calif. Fish Game 38: 485–498.

WESTRHEIM, S. J. 1958. On the biology of the Pacific ocean perch, *Sebastodes alutus* (Gilbert). M.Sc. Thesis. Univ. of Washington, Seattle, Wash. 106 p.

1967. Sampling research trawl catches at sea. J. Fish. Res. Board Can. 24: 1187–1202.

WESTRHEIM, S. J. 1970. Survey of rockfishes, especially Pacific ocean perch, in the northeast Pacific Ocean, 1963–66. J. Fish. Res. Board Can. 27: 1781–1809.

WESTRHEIM, S. J., AND J. A. THOMSON. 1970. Weight-length relationship for Pacific ocean perch (*Sebastes alutus*) collected off British Columbia in 1969. Fish. Res. Board Can. Tech. Rep. 237: 12 p.

Hunters, Trappers, and Gatherers of the Sea: a Comparative Study of Fishing Strategies

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Cove, J. J. 1973. Hunters, trappers, and gatherers of the sea: a comparative study of fishing strategies. J. Fish. Res. Board Can. 30: 249–259.

This study is a comparison of fishing strategies in three areas: the Newfoundland offshore fishery, the inshore salmon fishery of British Columbia, and an oyster fishery of Cornwall, England. From the Newfoundland fishery, a model was developed to account for differences in risk-taking by fishing captains. The model specified relations between reward structure, technology, and environment that influence captains' evaluations and, hence, decision-making.

The model was then tested in the other fishing contexts. The results indicate that the situational approach to risk-taking can be used for the problem of understanding short-term production strategies. This particular model, however, requires the addition of an historical dimension in order to adequately account for decision-making in all three fisheries.

Cove, J. J. 1973. Hunters, trappers, and gatherers of the sea: a comparative study of fishing strategies. J. Fish. Res. Board Can. 30: 249–259.

Dans le présent article, l'auteur compare les stratégies de pêche dans trois domaines — la pêche hauturière à Terre-Neuve, la pêche côtière du saumon en Colombie-Britannique et l'exploitation des huîtres dans le comté de Cornouailles, Angleterre. Dans le cas de la pêche à Terre-Neuve, il élabore un modèle qui tient compte des différences de risques que prennent les patrons de pêche. Le modèle précise les relations entre les modalités de bénifices, la technologie et l'environnement, facteurs qui influent sur les évaluations et partant, les décisions du capitaine.

Il applique ensuite ce modèle aux autres contextes de pêche. Les résultats indiquent que l'approche topique à une prise de risques permet de mieux comprendre les stratégies de production à court terme. Cependant, le modèle doit s'adjoindre une dimension historique s'il veut expliquer adéquatement le processus de décision dans les trois types de pêche.

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The effectiveness and control of man's exploitation on fish populations is generally seen in terms of technology and resource. An additional dimension that should be considered is motivation and its influence on strategies of exploitation.

The following is a comparison of fishing strategies in three fisheries. The decision makers compared were Newfoundland trawler captains, British Columbia seiner captains, and oyster dredgermen of the Fal River in Cornwall, England. In these fisheries, fishermen have a common problem of operating under conditions of high uncertainty. Considerable emphasis is therefore placed on planning. Two characteristics of that planning are short-term commitment and a large element of risk.

A situational focus was used to build a model of fishing contexts to account for the observed differences in strategies (Kogan and Wallach 1967, p. 123; Ortiz 1967, p. 197). The captains were considered in terms of their locations in a system to see how the structure of that system might influence their choices in different ways. Their locations were assumed to be loci of three major elements generally used in decision models: utilities, capabilities, and uncertainty. How these elements were structured and how the captains fitted into that structure is used for explanation.

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Newfoundland Offshore Fishery

The fleet examined was based in a town on the Burin Peninsula in Newfoundland. Since its settlement in 1811, the community's history has been closely linked to the fishing industry. Until the middle of the 1950's it was involved in the salt cod markets of the United States and the Mediterranean countries (Gerhardsen and Gertenbach 1949, p. 66–69).

By the beginning of the 1950's the demand for salt cod was diminishing. The new market for

fresh and frozen fish in the United States required quantities of fish and processing facilities that were not available locally. Outside capital investment was needed for entry into the market. It was provided by a foreign firm interested in establishing a plant in Newfoundland in conjunction with the provincial government's program for industrial development. The result was that the port was designated as a growth center, and an American firm built a modern processing plant and brought in offshore trawlers.

This change had implications for members of the community. Local control over production was replaced by outside control. The earlier paternalistic merchant/fisherman relation ceased to be important. In its place came wage labor involvement and a cash economy, with low security and low control for the individual fishermen.

By 1968 over 100 families had moved into the community, attracted by government relocation grants and job opportunities. An interesting distinction between land and sea based production was evident in that none of the fishermen were natives. Part of the reason for migrants working on the boats was that many had the necessary skills from having fished in Nova Scotia. Another factor was that work on land and sea took on status and reference group connotations.

Since offshore trawling requires extended periods at sea, differences in life style between fishermen and plant workers emerged. In general, fishermen were much less involved in home and community life. Being outsiders contributed to making fishermen members of an occupational subculture. As a result of this separation, much of our understanding of fishing as an occupation, and the problem of fishing strategies, can be achieved without reference to the wider social system. Consequently, it is argued that the fleet, the boats, and the environment are the major parameters for the research problem.

The Firm and Relations with the Fleet

Regulation of fish intake was one of the crucial problems for the plant, since it involved coordination of boats, production facilities, and available labor. One of the policies instituted by the manager was an obligatory "daily hail" from each boat. The purpose of the radio call was to report each boat's catch to date so that landing times could be coordinated.

The attempt to coordinate landings led to conflicts between management and captains. To be called in early was regarded by the captains as inhibiting their objective of maximizing catches, with the result that captains grossly underestimated total tallies so that they could stay out longer, unless their boats were near capacity. Underestimation was generalized enough that the plant manager assumed his captains would always lie, "I add 50,000 pounds to whatever they say after their second day out." Even then, he seldom received accurate information. I asked him to keep a record of hails and his estimates on the two trips that I made and he was right for only 3 of 14 tallies.

During the summer months the coordination problem was most severe. The plant bought fish from inshore fishermen as well as processing catches from its own trawlers. On three occasions in the summer of 1968, conflict occurred over which source of supply should be given priority. Due to inaccurate hails the manager had promised to purchase from some of the inshore fishermen. In one case, a portion of one trawler's catch was thrown into the fishmeal plant as a result.

For the trawlermen such conflicts created major problems. Virtually all of the rewards from fishing are determined by landed value of catch. The fact that a boat might be called in early or receive fishmeal prices for catch affected overall performance and rewards.

Fishing followed the traditional pattern of coadventure. Fishermen are viewed as entering into a short-term partnership with the boat owners. The owners invest their boats and gear and the men invest time and labor. The return for both parties is a fixed share of the landed value of catch.

Coadventure and company ownership of vessels has a number of implications for the fishermen. With the share system there are no income guarantees. It is possible for men to go out fishing for two weeks and return owing for food. In 1968 monthly incomes for deckhands ranged from $40.00 to $400.00.

Job security is also low since there are no contracts between owners and crew. By tradition the owners choose the captain and the captain selects the crew. A captain obtains job security by building a reputation as a fish catcher. In the company's view his worth is determined almost exclusively by the value of the catch that he lands. A captain is seen as "good" in relation to the performance of other captains in the fleet. At the end of each year the company totals the value of catch landed by each boat. The top performer becomes the fleet "highliner" and all other captains are ranked relative to his performance. A captain whose record is significantly lower than the others would probably be replaced.

A captain's performance is important in another way. The company uses relative catch in its assignment of boats. With the introduction of stern trawlers to the fleet, inequalities in capability were structured into the situation.

Finally, relative performance is one of the criteria used by fishermen in evaluating boats. Since crew are free to sign on and off any vessel, captains must compete for crew. Since income is on a share basis, high-performance captains generally have stable crews. Differences in crew stability tend to give some captains in the fleet an advantage in labor. A review of company records for 1967–68 indicated that the highliner had to replace only one man. In the same period the fifth ranking captain had a turnover of 25 men.

The company's emphasis on relative performance led to a highly competitive situation for captains. Not only income, but also prestige, job security, boat assign-

ment, and crew stability were determined primarily by landed catch.

Trawlers

By 1968 the fleet consisted of six boats, four side trawlers, and two stern trawlers. Although the work cycle is similar for both the stern and side trawler, the time required for completion differs markedly. On a side trawler, shooting and taking in requires an additional 15 min due to less powerful winches. The work cycles for various quantities of fish on the two types of boat showed significant differences in nonproductive time. Unloading time is longer for the side trawler. Only one bag (tied-off codend) can be taken on deck at a time. If more than 3000 lb of fish are caught in one tow, then the codend must be lowered into the water and filled by raising the rest of the net. On a stern trawler the problem is eliminated by taking the whole net over the stern in a single operation. For catches of over 3000 lb, the side trawler takes an additional 20 min per codend.

The stern trawler has other advantages that affect capability. It generally has a higher cruising speed, which means less travel time to and from the grounds, and a larger carrying capacity. Given a competitive situation, it is clear that a stern trawler captain has a definite edge. It will be argued later that this difference in fishing capability influences decisions about how and where fishing is done.

Environment and Resources

The three species fished are Atlantic cod, American plaice, and redfish. Cod is the most important commercially, accounting for 75% of total landings. All three species range over a wide area. Cod and plaice tend to be found off the Labrador coast and eastern Newfoundland. Redfish tend to be in the warmer waters of the Gulf of St. Lawrence and the southern Grand Bank.

The search for productive fishing locations is confounded by the size of fishing grounds and lack of information. There are no natural signs that can be used to locate fish schools and available technology did not reduce the uncertainty significantly.

The competitive nature of the fleet prohibits sharing of information. The only case of information exchange that I could discover was between captains of different fleets.

The lack of accurate information about fish locations is coupled with another characteristic of the resource, lack of effective control (Christy and Scott 1965, p. 6). The fact that trawling requires movement in order to effectively exploit a migratory resource means that no one boat or group of boats can control that resource.

The lack of information and control have implications for the choices a captain must make. Without precise information a captain is never sure about how he is doing relative to other boats and other locations. If a productive spot is found, a captain cannot keep others from sharing it except by withholding information.

Fishing Strategies

The crew of a trawler generally consists of 13 men. It includes the captain, mate, bosun, chief, and second engineer, cook, third hand, and deckhands. Their activities are organized around four functional areas: wheelhouse, deck, galley, and engine room.

The captain's domain is the wheelhouse, the information center for the vessel. Much of a captain's authority is based on his control over information and information resources (Barth 1966, p. 6–9). By tradition the captain is supposed to be the ultimate authority while at sea. In practice it appeared that captains restrict their use of authority to selection of fishing locations and how gear will be used (towing time and direction of tow).

Given the hundreds of square miles that could be fished, selection is the major problem. Preliminary choice of fishing ground is made on the basis of past experience. Other factors such as marketability and price are also taken into consideration.

Both captains that I observed knew what species would be most plentiful, and in which areas they would likely be found. They differed in their initial selection of fishing ground and species. The side trawler captain decided to fish in the Gulf of St. Lawrence for redfish. The other, captain of a stern trawler, opted for the Grand Banks and plaice.

On questioning them about their choices, the side trawler captain based his decision on the knowledge that redfish were more plentiful than plaice, in fact enough so that expected catch would make up for lower price per pound. The other captain agreed with the evaluation but argued that redfish were sometimes not landable. Since demand for plaice was greater, in glut situations the plant would not process redfish. As it turned out, the risk was real. With 220,000 lb of redfish aboard, the side trawler captain was told that none of the firm's plants in the area wanted redfish. Only an accidental delay in landing date changed the conditions.

On both the Grand Banks and in the Gulf of St. Lawrence the captains had to decide where to shoot their trawls. Oceanographic charts and navigational aids were used to determine areas with proper depth and bottom conditions. Actual fishing locations were decided by other sources of information.

The captain of the side trawler relied primarily on the fishfinder function of his sounders. In using sounder readings the captain had to evaluate highly ambiguous information. The types of readings provided were: (1) a dense mark high above the bottom line, which meant a school too far off bottom to catch with an otter trawl; (2) a thin mark just off the bottom line, which meant a small school of catchable fish; and (3) no reading, which meant either no fish or a large school so close to the bottom that the sounder could not discriminate.

In using these readings the captain looked for locations with type 3 readings. This meant that most tows were made over spots with either very low or very high yields. Catches for that trip ranged from 1000 to 20,000 lb.

The captain of the stern trawler thought that the fishfinder function was useless and kept it turned off for the entire trip. He relied on locations of other boats in making his selections. He argued that a clustering of

boats must mean moderately good yields or they would leave.

In addition, the stern trawler captain spent considerable time monitoring radio conversations between boats. Even though he knew that other captains would not deliberately release information about productive areas, he believed that one could often work out catches and locations. For part of that trip he was able to work with a friend from another fleet. They exchanged coded information about size of catches and locations, thereby increasing knowledge of alternatives without loss of fishing time. As could be anticipated, his range of catches was fairly consistent, between 3000 and 9000 lb per set.

It can be concluded that the two captains differed in their fishing strategies. The captain of the side trawler was a high risk taker, while the captain of the stern trawler used a low risk strategy. The risks taken also included real as well as opportunity costs. The stern trawler captain avoided areas with rocky bottom since there was a danger of tearing the trawl. The high risk captain made a number of sets on bad bottom, his rationale being that a good catch would lift the net off the bottom and avoid the rocks.

The risks taken were not limited to landed catch. Crew evaluation of captains included the kinds of decisions that were made. Fish catches are seldom seen as high or low in an absolute sense. Without information about yields in other areas crews tended to make their judgments on other grounds. Fishing in locations where there were other boats provided some rationale for a captain's decision. Even if catches were poor, they would be explained away as bad luck; the location itself was not questioned. Fishing alone did not provide such rationalization. The side trawler's crew were constantly complaining about the locations we were in. The absence of other boats was seen as evidence that the skipper had made a mistake. Even though we had a successful trip in terms of catch, four deckhands refused to go out on the following trip.

In summary, the context for the trawler captains had three characteristics: (1) a high degree of competition generated by the structure of rewards; (2) marked differences in capability due to the mixture of stern and side trawlers in the same fleet; and (3) a high level of uncertainty due to the extent of the fishing grounds and the species sought.

Model and Transformations

From the Newfoundland context, three elements were isolated that are important for understanding the research problem. Each of these elements can be seen to influence risk-taking propensity. A model was developed which combines these elements to account for differences in the observed fishing strategies. An expansion of that model can be made to predict expected patterns of decision-making in other contexts.

Model

Uncertainty refers to an awareness that choice alone will not produce a desired outcome. Factors external to the decision affect the actual outcome. For the Newfoundland captains, uncertainty is a result of the environment and species sought.

The costs of information search or exchange inhibit their use as mechanisms for reducing uncertainty. Search can be done only at the expense of fishing time. Sharing of information between boats is unlikely given the competitive characteristics of the fleet.

Risk-taking refers to potential loss. Applied to the findings from the Newfoundland case, it is apparent that uncertainty alone cannot account for differences in risk-taking. Given that the captain of the side trawler was consistently a high risk taker, other factors must be involved. The focal question is — why did he take high risks? The literature on decision-making would suggest that the outcome should be more important to that captain (Marschak 1964, p. 103–106). One approach to motivational differences is to look at how they link into the structure of rewards (Coleman 1962, p. 119–227). In the Newfoundland case, the reward structure is competitive. All rewards are a function of landed catch and, with the exception of income, are allocated by relative performance.

In the performance hierarchy the stern trawler captain was the company highliner, while the other was ranked fifth. Using a marginal utility assumption, it can be argued that the highliner captain would place less importance on additional units of catch beyond that needed to maintain his position. The only reward that could be increased by additional performance would be income and, with the highest earnings in the fleet, his marginal utility would be lower than that of the side trawler captain.

An implication of the analysis of motivation and structure of reward is that quantity of fish is directly related to risk-taking, which appears incongruous with the captains' positions in the hierarchy. Analysis of landings over a one-year period showed that, although the stern trawler had better total catch, its average per set was lower. In that year the stern trawler averaged 3700 lb/set, while the side trawler averaged 3900 lb/set.

The decisions made concerned use of time, labor, and equipment. The major determinant of capability was the trawler. As discussed earlier, the stern trawler had significantly less nonproductive time per unit catch. This difference in capability allowed the highliner captain to maintain his position without taking high risks. The fact that his boat could average more sets per trip more than made up for his smaller catches. In addition, the highliner had a more stable crew which gave him an extra advantage over the other captain.

Transformations and Predictions

At this stage the conclusions reached can only be regarded as ex post facto interpretations. If the model has any explanatory power it should be able to predict risk-taking in other fishing contexts.

The scope of the model can be increased by manipulating the original relations so that new combinations can be specified. It was argued that the situational factors generated conditions that influenced risk-taking in certain ways. By asking what the alternate situational factors might be, different conditions and effects on risk-taking can be isolated.

Size of fishing territory and species fished were seen as generating a high level of uncertainty in the Newfoundland offshore fishery. If the area fished were smaller, then the range of alternatives would be less. If the species sought provided signs, fishermen would have information for evaluating alternatives. Similarly, if the species were less mobile, then information needs would decrease. From these alternate environmental and resource characteristics a range of fisheries can be formulated and an ordering of uncertainty levels made:

Territory	Species	Levels of uncertainty
restricted	sedentary	1
restricted	mobile/nonmigratory	2
restricted	migratory	3
extensive	sedentary	4
extensive	mobile/nonmigratory	5
extensive	migratory	6

Competitive reward structure was argued to produce differences in motivation that could be measured by position in the performance hierarchy. If other reward structures and criteria were used to allocate rewards (e.g. seniority and status in the wider system), then expected motivation levels would be low for all members. If the reward structure were cooperative such that all members shared the rewards, then motivation levels would be high for all members. In fleets of the same type of boat and gear, capabilities would be more equally distributed.

How various combinations from the above relations should influence risk-taking can be determined after three assumptions are made: that each condition has equal weight, that their effects are additive, and that a decision-maker will prefer to take low risks.

A numerical value can be assigned to each state of the conditions. The value (1) will be given to states that induce high risk-taking and (0) to those that do not. Each combination of states can be thought to be a possible fishing context, or a location in one. Any empirical case which fits one of the combinations can be used to test the model. The predictions about risk-taking under each of the combinations are as given in Table 1.

British Columbia Salmon Fishery

Long before contact with white settlers, the indigenous Kwakiutl in the area to be discussed used the salmon resources as their major means of subsistence (Drucker 1955, p. 35–42). The Kwakiutl took advantage of the migratory pattern of the resource and fished the spawning rivers, using weirs and traps (Forde 1934, p. 76–79).

In the 1880's the first cannery was established in the area. For the Kwakiutl this had a number of implications (Gladstone 1953, p. 22–23). Fishing changed from a subsistence activity to a commercial activity. Technological and legislative developments in that commercial fishery led to traditional techniques and labor organization becoming obsolete. Finally, they were forced to compete with white settlers in the area, who had the necessary skills and capital to operate in the commercial fishery.

In 1936 the first all-Indian union was founded by the Kwakiutl (Gladstone 1953, p. 32). Its objective was to change their marginal position in the industry. However, this movement was not effective. The first significant development in changing Indian status occurred during World War II. High fish prices, lack of competition, and the possibility of buying Japanese boats confiscated by the govern-

TABLE 1. Expected effects of different combinations of uncertainty, motivation, and capability on risk-taking propensity.

Combination no.	Uncertainty state score	Motivation state score	Capability state score	Risk-taking
1	high (0)	high (1)	high (0)	low
2	high (0)	high (1)	equal (0)	low
3	high (0)	high (1)	low (1)	high
4	high (0)	low (0)	high (0)	low
5	high (0)	low (0)	equal (0)	low
6	high (0)	low (0)	low (1)	low
7	low (1)	high (1)	high (0)	high
8	low (1)	high (1)	equal (0)	high
9	low (1)	high (1)	low (1)	high
10	low (1)	low (0)	high (0)	low
11	low (1)	low (0)	equal (0)	low
12	low (1)	low (0)	low (1)	high

ment led to increased private ownership of boats and gear (Gladstone 1953, p. 34).

Contemporary Context

In 1969 the population of the community studied was 1800. The demographic composition was approximately half white and half Indian. Of this number, 200 were fishermen. Because of the large number of boats working out of the port, the study was restricted to the 35 seiners and their captains.

The Firms and Their Fleets

There were three processing companies with offices in the community. Although processing was no longer done locally, the firms still used it as a base during the salmon season. Managers and staff were sent to act as buyers and provide services for their boats.

One of the results of having three firms in the port was that there was little feeling of a total fleet. Seiner captains tended to compare their performance with other seiners fishing for the same firm. As one captain put it, "I'd like to be the best skipper on the west coast, but the important thing is to be better than anyone fishing for my company."

Not all of the seiners were company-owned. Some were owned outright by their captains and others were mortgaged through the processing firms. The companies had much the same control of their own boats as in Newfoundland. They picked captains for the season and assigned boats on the basis of past performance.

Owner captains were free of company control. Although not obligated to do so, many of them sold their catches to the processing firms rather than to small cash buyers. Advantages such as loans and use of company facilities led to long-term relations with the firms. Mortgaged boat çaptains were required to sell to the company holding that mortgage. However, relative performance was not crucial to their job security, as it was to hired captains.

Fishing was on a coadventure system; therefore, income was determined by landed value of catch. Major differences in captains' incomes resulted from differences in ownership. Owner captains received boat share and net share, as well as their own share.

There was no formal highliner system, though prestige was primarily determined by relative catch. One new captain said, "I really don't care how much I catch as long as I catch 50 more than the next guy." Relative performance was important for hired captains in that it influenced job security and boat assignment. For all captains, it was crucial in getting crew. Although men tended to stay on for the fishing season, a captain signing on qualified men required a good reputation as a fish catcher.

With one exception, relations between companies and captains were not conflicting. Without having to coordinate landings, company managers were concerned that each of their boats maximize production. Only one of the managers thought in terms of overall fleet performance and he hired captains solely from the Indian community, using traditional rivalries as a guide. He had found that hiring captains from the same band or kin group led to problems of control and performance. Captains recruited from the same band would frequently not want to outperform someone who ranked higher in the traditional system. Common membership could be used to create alliances against company authority, especially when replacing captains was concerned. The manager, therefore, made a point of hiring rivals in order to intensify competition and reduce alliance formation. He felt that it was sometimes better to hire a known poor performer than to hire someone who would bring the fleet average down.

In general, the structure of rewards was similar to that in Newfoundland due to the coadventure system and importance of relative performance. The major difference for motivation levels was ownership. It is assumed that owner captains are similar to high-ranking company captains, regardless of performance. Owner captains do not need top landings to keep their boats or jobs. Their incomes are higher due to additional shares. Only recruitment of crew is dependent on relative standing to any extent. Captains of mortgaged vessels, regardless of relative performance, would seem to be more like low-standing hired captains. As they are concerned with paying off loans on their boats, additional units of catch are important. Captains of mortgaged boats whom I got to know were all interested in paying off their loans as soon as possible.

Environment and Resources

The Pacific salmon are the primary resource base for the seiners. The regularity of their migrations means a degree of predictability about timing and relative size of annual runs. Captains know if any given year will be good, the size of runs being a function of the spawning year for the species.

An understanding of the environment and resource must take into account government regulation of the fishery. It could be argued that present technology is more of an adaptation to these regulations than it is to natural conditions. When fishermen are kept out of spawning rivers, for example, they are forced to search for runs in the straits. This requires highly mobile equipment, such as the seiner.

Since government decisions about fishing times and areas could only be made while the runs were on, frequently captains were not notified until late. The lack of long-range forecasting led to considerable hostility among fishermen. Many seiner captains felt that they dare not travel to distant areas because the area might not be open, which would mean lost time and cost of fuel.

The 1969 season was considered poor. Fishing "weeks" were often only 1-day long in order to insure adequate salmon escapements. As a result, most fishing was done within a 20-mile radius of the community.

Information about salmon is useful for determining fishing times and locations. Salmon only move with the current so, in slack tides, most seiners did not bother setting their nets. Salmon were thought to travel along the shores rather than in midstrait, hence, most fishing areas were close to shore. The presence of points or kelp

beds were thought to provide natural weirs that directed schools out to more open waters, therefore, boats tended to congregate in such areas.

One sign that indicated fish were present was the jumper. Salmon generally travel near the surface and occasionally will jump above it. While fishing, crew would spend considerable time looking for jumpers. It was not unusual for boats to go out a day or two before opening time, just to search for such signs. Unfortunately, jumpers were not a common occurrence. Seiner captains generally had to decide on fishing locations without such information, what they called fishing "blind." When fishing "blind" they were under constraints similar to those experienced by trawler captains. However, when jumpers were sighted, they operated under considerably less uncertainty.

In terms of environmental factors, it will be assumed that captains can operate under conditions of high and low uncertainty. The presence or absence of jumpers can be used as one indicator for determining that constraint.

Seiners

In 1969 crew structure on the 35 seiners was less complex than on the Newfoundland trawler. A typical crew consisted of a captain, cook, skiffmen, and deckhands. Most of the crew would perform more than one function — the captain would work on deck if required, one of the men would act as engineer, and the cook would work on deck or in the skiff.

Although the degree of specialization was less, the captain's primary role was that of decision-maker. The seine could be used as either hunting or trapping gear. When schools were sighted, the net could be set to enclose and capture. When fishing blind, it was set in expected paths of fish travel. As with trawler captains, the seiner captain had to decide on fishing locations and how the net would be set.

A major difference in crew relations was the relative lack of authority that the seiner captain had. On all the boats that I observed the captain was called by his first name. The crews felt free to be on the bridge and even criticize fishing locations. On two occasions crewmen refused to make a set because they did not agree with the captain, and in neither case was anything done. Barth (1966, p. 7–8) has argued that such informality and equality is a result of crew access to information and the requirement for cooperative effort. On seiners crew have the same information as captains about locations. The bridge is not an information center in the way it is on trawlers. More importantly, with a floating net, speed in retrieval is crucial. A captain has to rely on his crew to make decisions themselves; if they had to wait for orders, the lag might lead to gear damage or loss of catch.

As with trawlers, the seiners differed in capability. The most significant difference was between drum and powerblock gear. Drum seiners could complete a set in 35 min, while a powerblock took 50 min. It was, therefore, possible for a drum seiner to make twice as many sets per day.

The drum also permits a boat to get out of trouble more easily. The chances of the seine drifting under the boat or being caught on rocks is high. A change in wind or an error while taking-in can put a boat in serious trouble in seconds. Often only quick net retrieval will prevent serious damage.

The final major advantage of the drum seine is that fewer crew are needed. One drum seiner worked part of the 1969 season with only three men. Most powerblock seiners working the same territory had crews of five or six. Fewer men means more income per crewman.

A comparison of trawling and seining gear indicates two major areas of difference. The first is degree of visibility. In trawling it is virtually impossible to tell how the gear is set, or how much has been caught, when one is observing another boat. Since the seine is a floating net, visibility is higher. A captain moving into a new area will generally watch a few sets being made by others before deciding whether to stay. The newcomer can quickly tell if sets have to be made in any peculiar way, due to rocks or currents, and can find out how much is being caught.

The second difference is in degree of control. A seine is set rather than towed, therefore, two or more boats can dominate a productive spot. After one boat begins to close its net, a second boat can set around him. By taking turns in this manner, newcomers can be kept out. It is seldom worthwhile to wait until two or more boats have had their turn in order to get a chance to set.

Fishing Strategies

The seiner captain is faced with essentially the same problem as the trawler captain. He must decide among a range of alternative locations and how he will use his gear. The major difference is in precision. Seiners set on a spot rather than make a tow. The use of a floating net means more accuracy and a greater possibility for gear damage. Reefs, rocks, and currents are part of the environment. Setting too near such obstacles or making an inappropriate arc with the net can result in damage to the net and the boat.

The number of productive areas within 20 miles of the port are few, though there are spots in each area. All seiner captains are familiar with the alternatives but differ in the evaluations they make. Of 35 captains, I was able to observe and classify the decision-making of 25 over a period of 5 months. The criteria used, as to relevant combinations from the Newfoundland model, were:

Condition and level	Indicators
uncertainty (high)	no jumpers no other boats
uncertainty (low)	jumpers other boats
motivation (high)	mortgaged boat low rank in company
motivation (low)	private ownership of boat, high rank in company
capability (high)	drum seiner
capability (low)	powerblock seiner

I classified the decision-making of the captains using the following indicators:

High risk indicators	Low risk indicators
selection by largest amount ever caught	selection by average amount caught
setting near rocks, reefs, kelp beds	avoiding rocks, reefs, kelp beds
setting in strong currents and high winds	making half-sets (part of net) or avoiding strong currents or winds or both
fishing alone	taking turns

Rather than using the captains as units of analysis, I used the decisions. Classifying captains as to high or low risk would require arbitrary cutoffs, and the model specified propensities regardless of captain.

One way of viewing the problem is to say that the total number of observed decisions constitutes a population. The combinations derived from the Newfoundland case are sets of criteria for selecting samples. The proportion of high and low risks by captains in each of the combinations can be compared to the proportion in the total observations. If the two proportions differ in the predicted direction, then it can be assumed that the model tells more than we could have learned by simply making observations.

Since the classification of decisions is discrete, and the direction of risk-taking predicted, a binomial test was used (Siegel 1956, p. 41–42). The results of this test are:

Combination	Prediction	P	p	z	Significance
1	$P>p$	.51	.43	−.06	.05
3	$P<p$	.51	.66	4.9	.001
4	$P>p$	.51	.36	4.4	.001
7	$P<p$	.51	.76	3.5	.002
9	$P<p$	.51	.88	4.5	.001
10	$P>p$	.51	.50	−.3	.382

The British Columbia test indicates that the model has explanatory and predictive power. The results, however, are not as conclusive as they may appear. No random sampling of captains was possible, and generalizations would have to be restricted to that one season. The fact that captains assumed that the runs would be poor may have influenced decision-making in directions not covered in the model.

The results are suggestive for the assumptions underlying the model and transformations. Using Coleman's analysis for ordinal and attributional data (1964, p. 189–240), the proportions of variation accounted for are: uncertainty, 16%; motivation, 7%; and capability, 29%.

The above implies that the equal-weighting assumption is questionable. The 48% error factor indicates that the variables used are not sufficient. Since the major proportion of the error factor is in the direction of high risk, it would seem that captains cannot, or do not, operate on a pure low-risk strategy.

The additive assumption can also be questioned. Intuitively, the low weighting of motivation level seems inaccurate. Following Coleman (1964, p. 224–229), it was possible to examine for interaction effects among the dependent variables. It was found that motivation has a 1.7 intensifier effect on low capability and low uncertainty. Unfortunately, there was not sufficient data to determine the range of interactions.

Even with the above limitations, the data can be used to generate more precise predictions. The expected proportions of high risk-taking can be determined by the weightings given for each dependent variable, as shown in Table 2.

TABLE 2. Expected proportions of high risk-taking under different combinations of uncertainty, motivation, and capability.

Combination	Uncertainty	Motivation	Capability	Proportion high risk-taking
1	high	high	high	.55
2	high	high	equal	.55
3	high	high	low	.84
4	high	low	high	.48
5	high	low	equal	.48
6	high	low	low	.77
7	low	high	high	.71
8	low	high	equal	.71
9	low	high	low	1.00
10	low	low	high	.64
11	low	low	equal	.64
12	low	low	low	.93

Cornish Oyster Fishery

A major difference between the Fal River oyster fishery and those previously examined is the absence of technological development. The boats and gear used are nineteenth century in origin. In order to understand the relative lack of modernization in the fishery, and the contemporary orientations to production, emphasis is given to the history of the Cornish fisheries.

Historical Background

Prior to the thirteenth century, fishing was primarily a subsistence activity. The granting of an export licence to the Bayonne merchants resulted in the development of a commercial fishery. The charter given by King John opened a market for fish and provided access to salt, which was needed for processing (Jenkin 1932, p. 146). By the end of the fifteenth century, pilchard had become the major export of the county.

The first gear used in the pilchard fishery was a crude form of seine, worked from square-rigged vessels. This combination did not provide for efficient exploitation of the resource. The seine required fast vessels that could circle a moving school. Since square-rigged boats cannot be sailed against the wind, productive sets could seldom be made.

Pilchard tended to travel in large schools, rather than scattered as with Pacific salmon. This made "blind sets" impractical. Unless runs were sighted near the coast, boats would generally not go out.

A final problem was that the seine was open bottomed. Fish could swim under the net after being enclosed, which restricted setting to shallow waters. Since runs could occur as far offshore as 20 miles, it can be concluded that the seine was not well adapted to the resource.

In the seventeenth and eighteenth centuries, three innovations increased productivity in the fishery. Cornish fishermen came into contact with the French lugger (chassemarée), which was a type of fore-and-aft rigged boat (Hornell 1938, p. 40–42). Greater speed and ability to sail against the wind led to its adoption.

The second development was the three-net pilchard seine, which permitted faster sets and enclosure of larger schools. The seine unit consisted of three boats each working a special net — a main seine that was arced in front of the run, a stop seine that was set around the back of the school, and a tuck net that sectioned off part of the catch and prevented escapement (Jenkin 1932, p. 171–176). With this modification, the seine could be used as an effective piece of hunting gear.

The final innovation was the drift net. It is not clear if this was a local development or a diffusion, though it can be argued that the drift net did evolve from some type of seine (Von Brandt 1964, p. 164). Unlike the seine, a drift net does not have to be closed since it is an entangling technique. With the drift net, visibility of schools was not as important; nets could be set in expected paths of fish travel. It could also be used offshore since fish escapement was not a problem.

With expansion of the continental market to Mediterranean countries, demand for pilchard increased markedly. Except during periods of war, demand far exceeded supplies. The seasonal nature of the runs meant that fishing was intensive from August to November (Crouch 1935, p. 133). Other species such as herring and mackerel were sought in the remainder of the year, but their commercial value was considerably lower.

The schooling characteristic of pilchard led to a high level of competition between boats. One or two runs might be all that occurred in an area, hence, a major portion of a crew's livelihood for a year might be affected by how much was caught in a single day. Thomas (1893, p. 85–86) describes one set made off the coast of Falmouth in which 18 million fish were caught.

The competitive element was intensified by the gear used. Seine fishermen complained that drift nets blocked or broke up runs so that seines could not be used effectively. Similarly, the fact that drift nets could be left out indefinitely meant that they could control an area. Drift net fishermen argued that seining did not permit sharing in runs. A single seine unit could catch an entire school since the nets were often over half a mile in length. Periods of open conflict between seiners and drifters were common, which were only partially managed by agreements over fishing times (Fox 1943, p. 22–23; Gibson 1949, p. 18–21).

The size of catches caused periodic large inputs to the pilchard stations, which did the curing and exporting. Limitations in processing facilities and labor frequently led to an inability to buy catches in peak periods. Even with the development of bulk-curing techniques, glut situations did occur (Fox 1943, p. 25). Since catches could not be stored for more than a few days without rotting, fishermen were put in a position of competing for access to market. One strategy used by seining units was to get buyers to invest in nets, thereby insuring priority in landings (Rowe 1953, p. 30–36).

In 1927 the export of pilchard from Cornwall ended. Over-exploitation of the resource led to collapse of the industry. The only three regulatory influences that could have curbed the decline were market demand, regulation of the fishery, or an awareness on the part of fishermen that the resource was being over-fished. As previously mentioned, the market was expanding, and prices went up as supply decreased. The fact that pilchard runs were known not to come near the coast in certain years (Noall 1970, p. 14) did much to inhibit concern. Even when the fishery was over, most fishermen thought that offshore trawling for ground fish, rather than overfishing, was the cause of the decline.

The oyster beds of the Fal River did not become commercially important until the end of the nineteenth century. Due to lack of rapid transportation, urban markets such as London were not accessible.

The London market for oysters in the nineteenth century was dominated by the Whitstable dredgermen. Overworking of the Thames beds led them to search for new sources. In the 1840's, Essex boats began to visit the Fal for spat which could be relaid on the Thames (Anon. 1846, p. 5).

The towns of Truro and Falmouth regarded the Whitstable fishermen as a source of revenue. By an act

of Parliament, the towns were given rights over the intertidal zones of the estuary which included most of the oyster beds (Anon. 1853, p. 6). In 1869 the Truro town council established an Oyster Fishery Committee to regulate its portion of the beds (Anon. 1870, p. 8). The Committee placed restrictions on dredging during spawning seasons and set up licensing limitations (Philpotts 1891, p. 982). The fact that the resource was regulated was important for the later commercial development of the fishery.

For many Fal fishermen, oyster beds provided an alternative to the declining pilchard fishery. Movement into that resource base, however, required a number of adaptations. The boats and gear used for pilchard were unsuitable for the new environmental conditions. In place of the seine and drift net, the dredge became the main piece of gear. Unlike seining or drifting, dredging required that the boat be kept in motion. Towing from a moving boat is the easiest way to work a dredge, with the limitation that speed be kept to a minimum to prevent skipping. The sailing technique that evolved to keep speed down was to let the boat drift rather than attempt to increase headway.

The speed requirement and restricted fishing area led to a selection for certain hull designs. A comparison of licences issued by Truro in 1921 and 1961 indicate a selection process for boats with an overall length between 29 and 38 ft.

These boats had another characteristic in common, that of shallow draft. This was important not only for maximizing drift, but also for working in an intertidal zone. Current estimates show that a difference of 2 ft in draft represents an ability to work over 200 additional acres at low tide.

Sailing in the narrow confines of the Fal required a highly maneuverable boat. The lug sail was not well suited since the lug had to be lowered and placed on the other side of the mast when making major course changes. The frequency of such changes made the lug sail undesirable. In addition, variation in wind and tide required a sail that could be changed quickly.

The rig adopted by oyster dredgermen was the gaff rig. The gaff mainsail in combination with double headsails provided the handling speed and flexibility required. The contemporary gaff-rigged cutter is regarded by local fishermen as the best possible adaptation to local conditions. It can be sailed and worked by one man. The sail area can be changed to suit virtually all weather conditions. When properly trimmed, the boat sails itself for extended periods while the crew works the dredges.

One of the more obvious changes resulting from the move into oyster fishing was a decrease in crew size. The use of dredges did not require cooperative labor and the boats can be sailed single-handed. Capital investment in boats was low since most were already owned.

The major advantage, aside from companionship, of larger crews was increased catch. With a system of one share for the boat and one for each man, extra men meant more income.

Crew was limited to a maximum of two, however, with the size of boats used. Larger crews would require larger boats, which would lower productivity of the unit under the environmental limitations.

Unlike the pilchard fishery, dredging has few competitive elements. The sedentary nature of the resource means there is little problem with where the resource would be. The amount caught by any one boat was not to the immediate cost of others. The required movement of boats means that there can be no effective control over location. A "drift" over a productive spot cannot fish out that area. The path dredged is only 24 inches across and it is estimated that less than 30% of the oysters in the path of a dredge are picked up in one tow (Medcof 1961, p. 115).

Access to market was never problematic. The Cornish beds from their start were the last natural oyster fishery of any size in Britain. Even when quantities caught exceeded immediate demands, oysters could be stored and kept indefinitely. The buyers did not have to process catches and had storage tanks and relay beds.

A high performance orientation appeared to carry over from the pilchard fishery despite the above characteristics. By the early 1930's over half of the beds were fished out. In 1936 the Truro council introduced additional legislation to protect remaining beds. The Fishery Order of 1936 stipulated fishing times, size of oysters to be marketed, closed seasons, and the prohibition of motorized vessels. The final restriction was aimed at technological developments that would have led to more efficient exploitation.

Contemporary Fishery

In 1970–71, the Fal River oyster fishery appeared to be unchanged from the 1930's. The majority of the boats used were gaff-rigged cutters, with a few dredgermen working from smaller row boats (wink boats).

It was apparent that fishing out a large portion of the beds in the first decade of commercial activity had led to changes in orientation. There were strong group norms in the fleet about productivity. Dredgermen working out of one village generally met every morning to discuss fishing conditions. As a rule, unless weather and tide were ideal they would not go out. In 2 months that I spent with that group, they spent less than 60% of possible fishing time on the grounds. Going out late, returning early, and skipping Saturday were normal patterns.

When the oyster buyer visited, everyone would watch the amounts sold by each boat. If a boat had a catch that was higher than the group average there would be considerable comment. In another village, two newcomers were warned by older fishermen not to overwork. When they did not conform, they were told to leave the river and did.

Fishermen were willing to discuss the reasons for their emphasis on low productivity. They felt that the beds were going to be worked out regardless of what they did, but "taking it easy" would preserve them longer. They knew that restocking and cleaning the beds could revive the fishery, but refused to do so. The only apparent rationale was a realization that the fishery was not attracting younger men and would die out anyway. The conservationist attitude was also evident in their attitudes to innovation. Within the limitations of the Fishery Order there was considerable room for tech-

nological development. For example, dredgermen knew that their gear was inefficient, yet made no attempt to improve the design. Even though a boat owner could rank his dredges in terms of performance, there was no effort to standardize on the better ones.

Similarly, most of the fishermen were active sailing enthusiasts and were familiar with modern hull designs, sails, and equipment. Again, there was only one known attempt to apply such knowledge to the workboats. One boat owner tried a Gunter and a Bermudian mainsail. His project was undermined by the racing committee that governed the work boat races in the summer. He was told that, if he wanted to use anything other than a gaffer, he would not be permitted to race.

Work boat racing is an important part of the dredgermen's lives. During the summer closure there are weekly races and informal racing is carried out all year. Status in the fleet was based primarily on seamanship, which was demonstrated by standings in racing. It acted as a mechanism to inhibit maximum production in the fishery by channelling out motivation to the racing sphere and by restricting technological change.

After 8 months of research in the Fal River, I came to the conclusion that my model was inapplicable. I could place fishermen along my three dimensions and predict on their risk-taking. However, the model did not fit the context. All captains seemed to be low risk takers for historical rather than contemporary reasons. The knowledge that maximum performance orientation had led to the end of one fishery, and almost destroyed another, made that orientation unacceptable. All attitudes to production were conservationist and social relations tended to demand conformity to that principle.

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ANON. 1846. West Briton. Falmouth. July 3.
1853. Royal Cornwall Gazette. Truro. Sept. 2.
1870. Royal Cornwall Gazette. Truro. Dec. 24.
1967. Office consolidation of the British Columbia fishery regulations. Dep. Fish., Ottawa, Ont.

BARTH, F. 1966. Models of social organization. Roy. Anthropol. Inst. Occas. Pap. 23: 1–33.

CHRISTY, F., AND A. SCOTT. 1965. The common wealth in ocean fisheries. Johns Hopkins Press, Baltimore, Md. 281 p.

COLEMAN, J. 1962. Reward structure and the allocation of effort, p. 119–132. *In* J. Criswell [ed.] Mathematical methods in small group processes. Stanford University Press, Stanford, Calif.
1964. Introduction to mathematical sociology. The Free Press of Glencoe, New York, N.Y. 554 p.

CROUCH, J. 1935. Essay on the natural history of the pilchard. Roy. Corn. Polytech. Soc. 3: 65–107.

DRUCKER, P. 1955. Indians of the northwest coast. The Natural History Press, Garden City, New York, N.Y. 224 p.

FORDE, D. 1934. Habitat, economy and society. Metheun, London. 500 p.

FOX, C. 1943. The present position of the Cornish pilchard industry. Roy. Corn. Polytech. Soc. 3: 21–36.

GERHARDSEN, G., AND L. GERTENBACH. 1949. Salted cod and related species. FAO, Rome. 196 p.

GIBSON, J. F. 1949. Crabbing days. Corn. Rev. 1: 67–71.

GLADSTONE, P. 1953. Native Indians and the fishing industry of British Columbia. Can. J. Econ. Polit. 19: 20–34.

HORNELL, J. 1938. The fishing luggers of Hastings. Quart. J. Soc. Naut. Res. 24: 39–54.

JENKIN, A. 1932. Cornish seafarers. J. M. Dent, London. 195 p.

KOGAN, N., AND M. WALLACH. 1967. Risk taking as a function of the situation, the person and the group, p. 113–276. *In* T. Newcomb [ed.] New directions in psychology. Holt, Rinehart and Winston, New York, N.Y.

MARSCHAK, J. 1964. Rational behaviour, uncertain prospects, and measurable utility. Econometrica 18: 111–141.

MEDCOF, J. C. 1961. Oyster farming in the maritimes. Fish. Res. Board Can. Bull. 131: 158 p.

NOALL, C. 1970. Tales of the Cornish fishermen. Tor Mark Press, Truro. 46 p.

ORTIZ, S. 1967. The structure of decision-making among Indians of Columbia, p. 191–228. *In* R. Firth [ed.] Themes in economic anthropology. Tavistock Publications, London.

PHILPOTTS, J. R. 1891. Oysters and all about them. John Richardson, London. 374 p.

ROWE, J. 1953. Cornwall in the age of the industrial revolution. Liverpool University Press, Liverpool, U.K. 419 p.

SIEGEL, S. 1956. Nonparametric statistics for the behavioral sciences. McGraw-Hill Book Company, Inc., New York, N.Y. 312 p.

THOMAS, W. 1893. Romance of the Cornish cove. Houlston and Sons, London. 217 p.

VON BRANDT, A. 1964. Fish catching methods of the world. Fishing News (Books), London. 191 p.

Dispersed and Particulate Petroleum Residues in the Gulf of St. Lawrence

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LEVY, E. M., AND A. WALTON. 1973. Dispersed and particulate petroleum residues in the Gulf of St. Lawrence. J. Fish. Res. Board Can. 30: 261–267.

Concentrations of petroleum residues both as material in solution or dispersed through the water column and as fresh oil or tarry particles floating on the surface in the Gulf of St. Lawrence were measured during July–August 1971. Dissolved and dispersed forms were present in concentrations generally less than 5 μg/liter over the southern and western regions while 5–10 μg/liter were encountered in the north and east. A major source of these residues appears to be the Atlantic water that enters through Cabot Strait, and the distribution of these materials is closely related to the circulation of water within the Gulf. Surface oil was present at approximately 50% of the stations occupied in concentrations generally less than 100 μg/m² but as great as 12,400 μg/m² in the vicinity of the sunken barge *Whale*. There is no evidence to indicate an appreciable change in concentrations to those of a year ago.

LEVY, E. M., AND A. WALTON. 1973. Dispersed and particulate petroleum residues in the Gulf of St. Lawrence. J. Fish. Res. Board Can. 30: 261–267.

Au cours des mois de juillet et août 1971, nous avons mesuré les concentrations de résidus d'hydrocarbures soit en solution ou dispersés à travers la colonne d'eau, soit sous forme d'huile fraîche ou de particules goudronneuses flottant à la surface dans le golfe Saint-Laurent. Les résidus dissous et dispersés étaient présents en concentrations ordinairement inférieures à 5 μg/litre dans les régions sud et ouest, alors qu'au nord et à l'est, elles atteignaient 5–10 μg/litre. Une bonne partie de ces résidus semble provenir de l'eau atlantique qui pénètre dans le golfe par le détroit de Cabot, et la répartition du matériel est intimement liée à la circulation de l'eau dans le golfe. Il y avait de l'huile en surface à environ 50% des stations occupées, à des concentrations ordinairement inférieures à 100 μg/m², sauf aux environs de l'épave submergée du *Whale*, où elles atteignaient 12, 400μg/m². Les concentrations ne semblent pas avoir changé appréciablement par rapport à l'année précédente.

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DURING the past decade, it has been realized that repeated discharges of a variety of waste substances into the environment may have long-term ecological effects even in situations where there is no evidence of any immediate detrimental effect or where immediate effects appear to be of a localized or short-term nature.

Before the effects of discharges of hazardous substances into the marine environment can be assessed, reliable data must be obtained on background concentrations and their temporal variations in both the coastal and open ocean areas. The absence of information concerning levels of petroleum-derived pollutants and their transport in the ocean became obvious following the sinking of the tanker *Arrow* in Chedabucto Bay, Nova Scotia (Fig. 1), in February 1970 when most of its cargo of 16,000 metric tons of Bunker C fuel oil was released in the waters of the bay (Anon 1970). This incident provided an opportunity to study the impact of an injection of a large amount of residual fuel oil into a partially enclosed bay having ready access to the open ocean, and the effects such an injection might have on the background levels in the surrounding coastal and open ocean domains. Accordingly, a series of physical and chemical measurements were made of the effects of the *Arrow* spill on the concentrations of residual oils in Chedabucto Bay and its approaches and also in the open North Atlantic (Forrester 1971; Levy 1971). Subsequent measurements (Levy 1972; Gordon and Michalik 1971) indicated that the background concentrations of petroleum-derived substances in these regions had returned to "normal" during the year following the spill.

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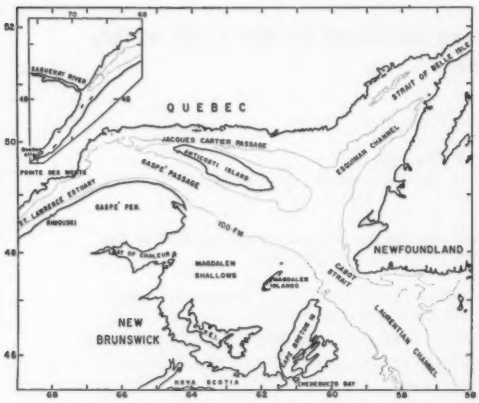

FIG. 1. Map of Gulf of St. Lawrence.

The first results from a detailed study of petroleum residues in the Gulf of St. Lawrence, obtained from a series of stations extending from Cabot Strait through the Gulf and estuary[1] and as far upriver as Montreal, showed average concentrations of about 2.3 µg/liter with no obvious relation between concentration and either location or depth. Somewhat higher concentrations (average 3.3 µg/liter) of petroleum residues were encountered in areas of high ship traffic (Levy 1971).

In September 1970 a new source for the input of petroleum residues into the Gulf of St. Lawrence was introduced when the barge *Whale*, carrying a cargo of 4000 metric tons of residual fuel oil, sank on the Magdalen shallows. The barge remained intact and only a small amount of oil was lost at the time of the sinking followed by a slow but persistent leak. To determine the impact of this incident a second cruise in the Gulf of St. Lawrence was made during July 12–August 8, 1971. During this cruise, the distribution of petroleum residues was studied at a network of 90 stations covering the entire region from the approaches to Cabot Strait to the Strait of Belle Isle, the Jacques Cartier Passage, the St. Lawrence estuary, Saguenay fjord, Gaspé Passage, Bay of Chaleur, and the Magdalen shallows (Fig. 1). In addition, an intensive study was made of the region in which the *Whale* incident occurred. This paper describes our attempts to evaluate the levels of petroleum residues in the Gulf of St. Lawrence in terms of their relation to man's activities and the oceanographic characteristics of the region.

[1]Following the terminology of Forrester (1967), the term "Gulf" is used in this paper to refer to the area eastward from Pointe des Monts bounded by the mainland, Cape Breton Island, and Newfoundland, whereas the term "estuary" pertains to the area between Pointe des Monts and Quebec City (Fig. 1).

Sampling and Analytical Procedures

Dissolved and Dispersed Oil

Water samples were collected in 12-liter Niskin samplers from just below the surface, mid-depth, and just above the bottom at the stations indicated in Fig. 2. Sampling in the *Whale* area was carried out at a group of stations arranged on concentric circles with radii of 1.9, 7.6, and 18.5 km extending outwards from the marker buoy that had been placed at the site of the sunken barge (47°25.0′N; 63°19.0′W).

Carbon tetrachloride extracts were taken from 1-liter subsamples of the sea water and the extracts returned to the laboratory for further processing and analysis by fluorescence spectrophotometry (Levy 1971). The excitation spectrum of each sample was scanned from 260 to 500 nm and the emission spectrum from 250 to 500 nm (excitation at 310 nm). Invariably, the dominating characteristics of the excitation and emission spectra were the peaks at 310 nm and 360 nm, respectively, features which are typical of the residual material in Bunker C oils. In a few instances, however, several small peaks superimposed on the spectrum of the residual oil suggested the presence of small amounts of other substances. In no case, however, was this material present in sufficient quantity to require any attempt to remove it from the sample.

The amount of residual oil present in the sample was estimated by comparing the intensity of the fluorescence at 360 nm to those of three members of a set of four standards containing 0.5, 1.0, 1.5, and 2.0 mg/liter of the Bunker C oil carried by the *Arrow*. In each case, the three standards having concentrations closest to that of the unknown sample were placed with the unknown in the four-cell compartment of the fluorescence spectrophotometer, and the emissions from the four samples measured under identical instrumental conditions. Because of the difficulty in reproducing exactly the slit widths, wavelength settings, electrical amplification, and other critical instrumental parameters, this procedure is superior to that of using a previously prepared calibration curve to relate emission intensity to concentration. Concentrations of 0.5 µg/liter in the seawater sample can be determined by this procedure.

Floating Particulate and Fresh Oil

The sea surface was sampled by towing a modified version (Levy and Walton 1971) of a neuston sampler (Sameoto and Jaroszynski 1969). Basically, the sampler consists of an open-ended aluminum box to which a nylon plankton net (mesh opening 243 µm) is attached. It is constructed for towing to the side of the ship so as to sample an area of the sea surface that has not been disturbed appreciably by the ship's passage. The device is towed for one nautical mile (1.9 km) at a speed, depending on sea conditions, of 5–7 knots (2.5–3.5 m/sec). Approximately 740 m^2 of sea surface are thereby sampled to a depth of 15–20 cm. A total of 45 stations

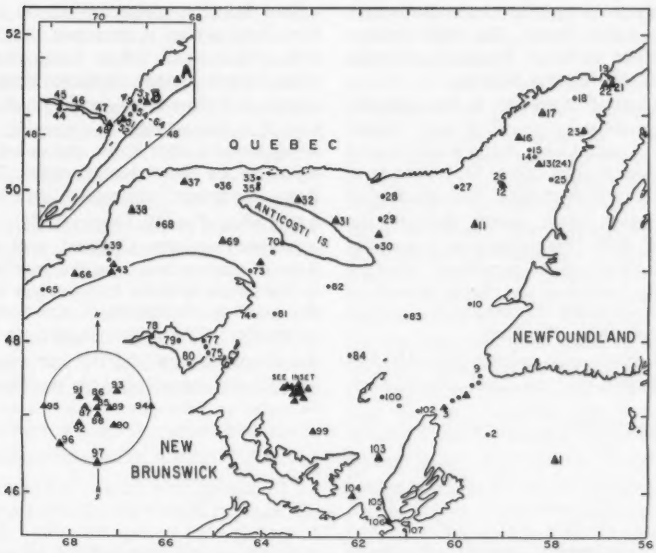

FIG. 2. Location of stations sampled in the Gulf of St. Lawrence. Samples for dissolved and dispersed petroleum residues were taken at all stations except A and B. Those marked with a triangle were also sampled for floating petroleum residues.

distributed throughout the Gulf (Fig. 2)[2] were studied using this device with particular attention given to the vicinity of the *Whale*.

The contents of the sampler generally consisted of a varied assortment of petroleum particles, neuston, small fish, terrestrial insects, fragments of seaweed, and other floating debris. Larger pieces of debris were washed with sea water and discarded. The remainder of the sample was frozen and returned to the laboratory for processing. Fresh oil, which adhered to the net, was removed with carbon tetrachloride.

Results and Discussion

VISUAL OBSERVATIONS

Oil was visible in three areas during the ideal weather conditions which prevailed throughout this cruise. In the first two, at the outer reaches of the estuary (Stations 41–43) and in the approaches to the Strait of Belle Isle (Station 20), thin slicks were present during calm mornings which followed nights of dense fog but disappeared as soon as the surface was agitated by the wind. A high density of shipping passes through both areas during the summer months and it is possible that ships in the area may have discharged their oily wastes shortly prior to our sampling period. The third area was downwind from the marker buoy that had been placed at the site where the *Whale* sank. During both days in which intensive sampling was carried out, oil was present in this area in a variety of forms including long rope-like chunks, pancake-like shapes, and both heavy and iridescent slicks. However, the rate at which the oil was leaking from the barge was not great, and oil was not visible on the surface at distances greater than 1.9 km from the marker buoy.

BACKGROUND LEVELS OF DISSOLVED AND DISPERSED PETROLEUM RESIDUES

The distributions of dissolved and dispersed petroleum residues in the water column just below the surface, at mid-depth, and near the bottom in the Gulf of St. Lawrence and its approaches during July–August 1971 from the 286 measurements carried out are shown in Fig. 3 A–C.

The background level throughout the water column at the two stations in the Atlantic waters outside the Gulf was 5–10 μg/liter, with localized con-

[2]This work is part of a much more extensive investigation of the distribution of petroleum residues floating on the surface of the open ocean that will be described in a forthcoming publication.

centrations as high as 18 μg/liter at the surface in the approaches to Cabot Strait. The high concentrations in the surface waters in this area are probably a consequence of shipping density.

Since the background observed in the Atlantic waters in the approaches to the Gulf were higher than those within, it would seem that a major source of petroleum residues in the Gulf of St. Lawrence is the Atlantic water which enters through Cabot Strait and to a much lesser extent through the Strait of Belle Isle. With this premise as a working hypothesis, the distribution of petroleum residues in the Gulf of St. Lawrence can be explained in terms of present knowledge concerning the general circulation of water within the Gulf.

On the basis of geostrophic calculations, McGregor (1956) has indicated that the circulation through Cabot Strait is characterized by an outward surface flow, which is strongest on the Cape Breton side, and a weak inflow along the Newfoundland side. Direct current measurements, however, have shown an inflow at all depths on the Newfoundland side (Lawrence 1968). As a result, the background levels in the Cabot Strait region are influenced considerably by the Atlantic waters. The deep waters flow "upstream" through Cabot Strait along the Laurentian Trough (Trites 1971), which branches into the Esquiman Channel, and carry concentrations of greater than 5 μg/liter northeastward almost to the Strait of Belle Isle. Surface concentrations in the extreme northeastern region of the Gulf were generally 5–10 μg/liter, suggesting an upwelling of the deeper waters and the intrusion at the surface of Atlantic waters through the Strait of Belle Isle.

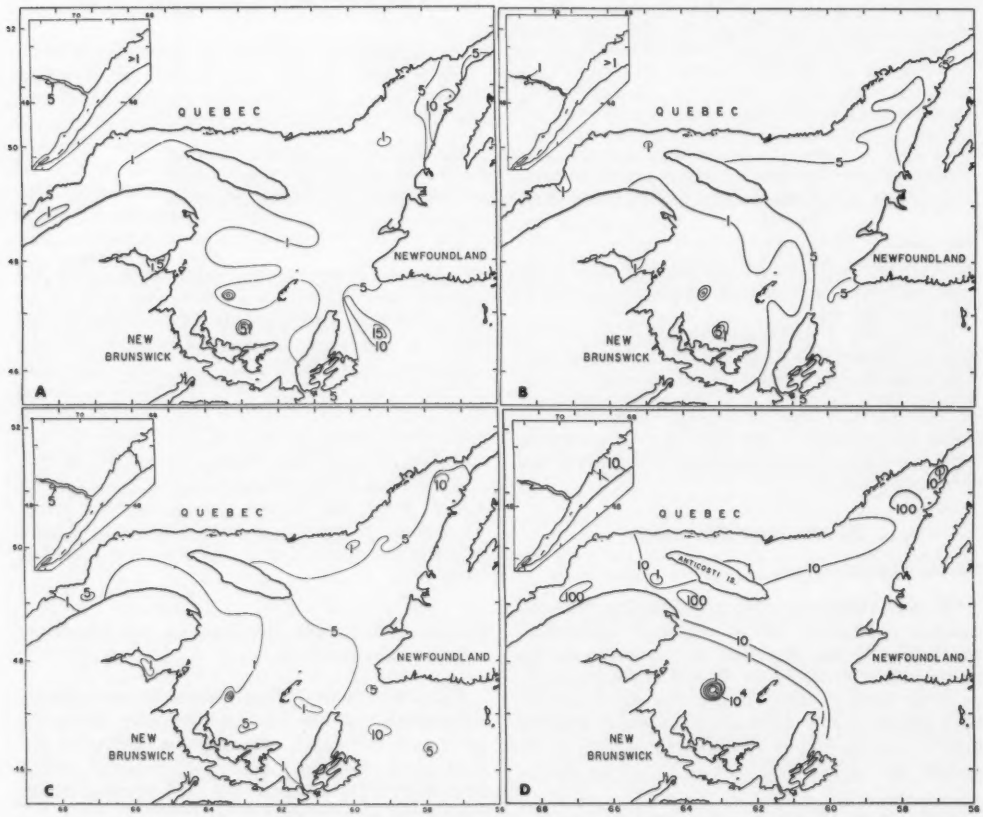

FIG. 3. Distribution (μg/liter) of petroleum residues in dissolved and dispersed forms at the surface (A), mid-depth (B), and bottom (C) of the Gulf of St. Lawrence and of particulate and "fresh" petroleum residues (μg/m²) floating on the surface (D) (July 12–August 8, 1971).

Although there is no large net flow through the Strait of Belle Isle, there is usually an inward movement of Labrador coastal water on the north side and a small outflow of Gulf water on the south. At times, however, a strong inward or outward flow dominates, depending on meteorological conditions inside and outside the Gulf (Farquharson and Bailey 1966). The effect of this intrusion of Atlantic water on the background level of petroleum residues on the surface of the northeastern corner of the Gulf is evident in Figure 3A. Alternatively, it might be surmised that the high surface values reflect the convergence of ship traffic which takes advantage of this shortcut to and from the Atlantic during the ice-free season. A somewhat lower background (1–2 μg/liter) was observed along the north shore of the Gulf and through the Jacques Cartier Passage as a result of the diluting effect of the fresh water entering from the unpolluted rivers that drain the wilderness area of northern Quebec.

By the time the flow along the west branch of the Laurentian Channel reaches the Gaspé Passage, the background levels in the deep waters have decreased to 1–5 μg/liter on the northeastern and to less than 1 μg/liter on the southwestern side of the Channel. A further decrease occurs as the waters proceed through the Estuary and approach the extremity of the trough near the mouth of the Saguenay River. While undergoing this journey from the Atlantic through Cabot Strait and along the Laurentian Channel to its termination, the background level of dissolved and dispersed petroleum residues in the deep waters decreased from 5–10 μg/liter to less than 1 μg/liter. It is interesting to note that dissolved oxygen concentrations decreased from 4.5–5.0 ml/liter to about 2.5 ml/liter as the deep waters passed from Cabot Strait to the Saguenay (Levy and Walton unpublished data). This decrease in oxygen concentration is probably a result primarily of biological processes. This biological activity is likely to lead to the degradation of petroleum residues and, hence, it is not surprising to observe this parallel behavior in dissolved oxygen and petroleum residue concentrations. At the upper reaches of the Laurentian Channel, there is an area of suspected upwelling where the petroleum and oxygen depleted deep waters rise and become mixed with the richer brackish waters flowing downstream from the inner Estuary and St. Lawrence River.[3] The combined effects of the outflow from the St. Lawrence River, the input from the high density of shipping which converges in this region, and the upwelling result in concentrations of 1–5 μg/liter in the seaward flowing upper layers. These concentrations prevailed throughout the estuary.

It is pertinent to note that the waters that flow from the St. Lawrence estuary over the sill (maximum depth 22 m) into the Saguenay fjord system carry with them a background of 1–5 μg/liter of petroleum residues. This background was observed throughout the Saguenay system except near the town of Port Alfred (Station 44) where a localized input, probably from domestic and pulp mill wastes, was evident. Salinity, temperature, and dissolved oxygen measurements made at this time revealed that the water below approximately 20 m within the Saguenay fjord system was very uniform with high oxygen concentrations (6.5–7.0 ml/liter) extending from the surface to the bottom. The dominating factor with respect to both the petroleum background level and the temperature, salinity, and concentration of dissolved oxygen appears to be the transport over the sill of St. Lawrence Estuary water into the fjord system and of Saguenay water out of it.

In the Pointe des Monts–Rimouski area, the seaward flow of the surface layers through the estuary develops into the Gaspé current which extends the entire length of the Gaspé coast (Trites 1971). On leaving the Gaspé area, this seaward flow tends to spread over the Magdalen shallows and, coupled with the inward flow on the Anticosti side of the Gaspé Passage (El-Sahb et al. 1969), constitutes an overall counterclockwise circulation of the surface waters in the central region of the Gulf southeast of Anticosti Island (Bumpus and Lauzier 1965). These circulation patterns result in the background level of approximately 1 μg/liter observed over much of the Magdalen shallows. Much higher concentrations, however, were present in the vicinity of the *Whale* (Station 85) and at one location north of Prince Edward Island (Station 99). The effect of the input from the *Whale*, however, appeared to be localized and had only a minimal effect on the general background levels of petroleum residues in the Gulf of St. Lawrence at the time of this survey. The intricate pattern observed in the surface waters of the central Gulf appears to be a direct result of oil that was leaking from the *Whale* being transported away by the wind and the complex surface circulation in the area. At Station 99 concentrations significantly greater than the surrounding background level were observed at all depths, suggesting the possible presence of a local source such as a seep or a wreck.

The waters off the northwestern shore of Cape Breton Island (El-Sahb et al. 1969) are character-

[3]Although sampling was not carried out beyond the Saguenay during this survey, data collected in 1970 indicated slightly higher background levels between Montreal and the mouth of the Saguenay than in the lower estuary or the Gulf (Levy 1971).

ized by a concentration that is intermediate between that of the water over the Magdalen shallows and that which enters from the Atlantic.

Although the sampling program did not permit the waters leaving the Gulf to be traced beyond Cabot Strait, the outflow from the Gulf of St. Lawrence passes around the northeastern part of Cape Breton Island and then branches with one arm flowing eastward and the other in a southwesterly direction parallel to the coast of Nova Scotia (Bumpus and Lauzier 1965). McLellan (1954) has shown that the upper 100 m over the Scotian shelf are dominated by the Gulf of St. Lawrence water mixed with varying proportions of slope waters, while deeper waters are a mixture of slope and Labrador current waters. If the waters over the continental shelf off Nova Scotia are dominated by the outflow from the Gulf of St. Lawrence, with a general background of less than 5 µg/liter, this accounts for the background of 0.2–3.2 µg/liter observed off the coast of Nova Scotia in January 1971 after the effects from the input from the *Arrow* incident had subsided (Levy 1972). Although a background of 5–10 µg/liter was observed in the Atlantic south of Newfoundland during the present study, this is not incompatible with the earlier observations as the more northern waters become diluted by the outflow from the Gulf before passing over the continental shelf off the east coast of Nova Scotia. A more extensive study of background levels of petroleum residues in the western North Atlantic is, however, required. Besides establishing much-needed background levels, such a study would provide valuable information concerning circulation and mixing of water masses since, on the basis of the present work, the petroleum background gives some useful information on water movements.

Petroleum Residues in Floating Forms

The distribution of petroleum residues as relatively nonsticky particles and as "fresh oil" floating on the surface is indicated in Fig. 3D. No indication of oil in either form was evident at 20 of these stations, while "fresh oil" adhered to the sampler at 9 and particles were collected at 23 of the stations.

The most obvious feature of these data is the extreme variability with values ranging from 0 at almost 50% of the stations to over 12 mg/m^2 at two stations in the vicinity of the *Whale* where fresh oil was abundantly present on the surface.[4] Although it is evident from these data that the particulate form of oil was much more widely distributed throughout the Gulf than the fresh, whenever a large amount of oil was encountered, it was invariably in the fresh form. The distribution of the total of both forms on the surface in the Gulf of St. Lawrence is indicated in Fig. 3D. The high values are directly related to man's activities in the region; namely, the leak from the *Whale* on the Magdalen shallows and the most likely discharge of waste oils associated with the ship traffic that converges at the entrance to the estuary and the approaches to the Strait of Belle Isle.

As was the case with the dissolved and dispersed residues, the lowest values for floating residues were observed over the Magdalen shallows and in the inner portion of the estuary. The low values in the estuary may be related to the greater caution exercised in discharging oily wastes in this area. Alternatively, the processes which lead to the removal of oil from this region may be sufficiently rapid and efficient to prevent the formation of tarry particles.

Over most of the surface of the Gulf, the general background was less than 50 µg/m^2 suggesting that the system is, to a large extent, "assimilating" present inputs. All regions and depths, however, must be measured periodically in the future to determine environmental changes in the area.

Comparison of Background Levels in 1970 and 1971

Background levels in 1970 ranged from 1.5 to 4.2 µg/liter in the Gulf and 2.3 to 5.5 µg/liter in the region between the Saguenay and Montreal with a concentration greater than 5 µg/liter at only one station (Levy 1971). However, in 1970, the area surveyed was primarily along the southern slope of the Laurentian Trough in the southern and western region of the Gulf and estuary where the background level in 1971 was less than 5 µg/liter. The northeastern regions, which had backgrounds exceeding 5 µg/liter in 1971, were not studied in 1970. Since it is possible that a similar situation prevailed during the previous year, there is not firm evidence to suggest an appreciable change in the background levels of petroleum residues between the summers of 1970 and 1971.

Conclusions

The notion that the input of petroleum residues from land drainage, domestic and industrial wastes,

[4]Since oil in particulate and fresh forms tends to be present on the surface, except when carried downwards by extraneous forces, it is assumed that the material collected was, in fact, on the surface at that time. It is appropriate, therefore, to express the mass of material collected in terms of the surface area sampled.

and other sources arising from man's activities in the Gulf of St. Lawrence region is a major source of the petroleum background levels in the Gulf has not been established. Indeed, the present study has demonstrated that the dominating factor appears to be the contribution of petroleum residues from waters that enter the Gulf system from the Atlantic Ocean. Present background levels are relatively low and there is no evidence for an increase in concentrations during the period July 1970 to July 1971. In some localities, higher-than-background concentrations are directly attributable to man's actions. Notable amongst these are the *Whale* incident and discharges from shipping.

In interpreting these conclusions two points must be borne in mind. High level pollution of the type that has immediate and obvious effects is not present in the Gulf of St. Lawrence at this time, except for a few restricted localities that have had little impact on the area as a whole. On the other hand, it is believed that prolonged exposure to low concentrations of certain pollutants may interfere with the reproduction and migration of marine organisms and transfer processes in the marine food web. Since the effects of chronic low-level pollution and their long-term repercussions are not well understood, this is an important area for research. Nevertheless, it is essential that, in the meantime, existing background levels of various pollutants be established so that changes in the near and distant future can be assessed.

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ANON. 1970. *The Arrow incident*. Report of the scientific coordination team to the head of the task force operation oil. Prepubl. ed., July 1970. Inf. Can. Cat. No. T22–2470/2.

BUMPUS, D. F., AND L. M. LAUZIER. 1965. Surface circulation on the continental shelf off eastern North America between Newfoundland and Florida. Ser. Atlas Mar. Environ. Amer. Geogr. Soc., New York, N.Y. Folio 7.

EL-SAHB, M. I., W. D. FORRESTER, AND O. M. JOHANNESSEN. 1969. Bibliography and some aspects of physical oceanography in the Gulf of St. Lawrence. McGill Univ. Mar. Sci. MS Rep. 14.

FARQUHARSON, W. I., AND W. B. BAILEY. 1966. Oceanographic study of Belle Isle Strait, 1963. Bedford Inst. Oceanogr., Dartmouth, N.S. MS Rep. 66(9) 78 p.

FORRESTER, W. D. 1967. Currents and geostrophic currents in the St. Lawrence estuary. Bedford Inst. Oceanogr. MS Rep. 67–5: 175 p.

1971. Distribution of suspended oil particles following the grounding of the tanker *Arrow*. J. Mar. Res. 29: 151–170.

GORDON, D. C., JR., AND P. A. MICHALIK. 1971. Concentration of Bunker C fuel oil in the waters of Chedabucto Bay, April 1971. J. Fish. Res. Board Can. 28: 1912–1914.

LAWRENCE, D. J. 1968. Current meter data from Cabot Strait, 1966. Atl. Oceanogr. Lab., Bedford Inst., BI Data Ser. 68(10–D).

LEVY, E. M. 1971. The presence of petroleum residues off the east coast of Nova Scotia, in the Gulf of St. Lawrence and the St. Lawrence River. Water Res. 5: 723–733.

1972. Evidence for the recovery of the waters off the east coast of Nova Scotia from the effects of a major oil spill. Water Air Soil Pollut. 1: 144–148.

LEVY, E. M., AND A. WALTON. 1971. An evaluation of two neuston samplers. Atl. Oceanogr. Lab., Bedford Inst. AOL Rep. 1971 9.

MCGREGOR, D. G. 1956. Currents and transport in Cabot Strait. J. Fish. Res. Board Can. 13: 435–448.

MCLELLAN, H. J. 1954. Temperature-salinity relations and mixing on the Scotian shelf. J. Fish. Res. Board Can. 11: 419–430.

SAMEOTO, D. D., AND L. O. JAROSZYNSKI. 1969. Otter surface sampler: a new neuston net. J. Fish. Res. Board Can. 25: 2240–2244.

TRITES, R. W. 1971. The Gulf as a physical oceanographic system. 2nd Gulf of St. Lawrence workshop, Bedford Inst., Nov. 30–Dec. 3, 1970. 168 p.

NOTES

An Initial Evaluation of Ethylene Oxide for the Sterilization of Formulated and Pelleted Fish Feeds

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TRUST, T. J., AND A. J. WOOD. 1973. An initial evaluation of ethylene oxide for the sterilization of formulated and pelleted fish feeds. J. Fish. Res. Board Can. 30: 269–274.

Satisfactory sterilization of dried pelleted fish foods was achieved with an ethylene oxide–carbon dioxide gas mixture (20:80). The growth rates of juvenile rainbow trout (*Salmo gairdneri*) fed on feed so sterilized were comparable to or better than those of trout fed the same feed not sterilized.

TRUST, T. J., AND A. J. WOOD. 1973. An initial evaluation of ethylene oxide for the sterilization of formulated and pelleted fish feeds. J. Fish. Res. Board Can. 30: 269–274.

Nous avons pu stériliser adéquatement de la nourriture à poisson en boulettes séchées en utilisant un mélange gazeux d'oxyde d'éthylène et de gaz carbonique (20:80). Le rythme de croissance de jeunes truites arc-en-ciel (*Salmo gairdneri*) auxquelles on donne une nourriture stérilisée de cette façon est égal ou supérieur à celui des truites recevant les mêmes boulettes non stérilisées.

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THE control of bacterial infections in fish populations maintained under intensive management conditions requires, amongst other things, that the water supply contain minimal numbers of bacteria, and that the feed used be free of bacteria. The bacterial burden carried by normal animal and human feedstuffs has been the subject of a number of investigations (Thatcher and Clark 1968). The quantitative and qualitative aspects of the microflora of fish feeds has also been examined (Trust 1971; Trust and Money 1972). These studies show that commercial fish feeds contain a mixed microflora including bacterial species potentially harmful to man and fish. The nature of this microflora is, in part, determined by the ingredients used in feed formulation. The elimination of this microflora subsequent to feed mixing would do much to lower the probability of feed-borne infection and, of equal importance, greatly facilitate the maintenance of sanitary conditions in hatcheries and rearing ponds, and in their effluents.

Success has attended the use of gaseous germicides for the dry, low temperature sterilization of particulate materials (Sykes 1967). Of the various possible germicides that have been used with foods and feeds, ethylene oxide appears to have met with the greatest success and appears to be the agent of choice (Wood 1941; Sykes 1967; Reddy et al. 1968; Coates 1969; Kereluk and Lloyd 1969).

This paper reports on an initial study on the effects of ethylene oxide treatment of pelleted dry fish feeds on the bacterial burden of the feeds, and also reports on the growth of juvenile rainbow trout (*Salmo gairdneri*) fed upon the ethylene oxide treated feeds.

Materials and methods — Three fish diets were used in this study.

Diet 1,[1] producing a slow growth rate, was formulated by this laboratory.

Diet 2,[2] producing a medium growth rate, and Diet 3,[3] yielding a rapid growth rate, were commercial diets.

The pellets were produced by extrusion through a $\frac{1}{16}$-inch die of a California Pellet Mill. Four commercial feedstuffs, bran, turkey grower pellets, duck grower pellets, and chick starter pellets, were examined. These

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pelleted feedstuffs were treated with ethylene oxide. Pre- and post-treatment samples were submitted to bacteriological examination and to bioassay with rainbow trout.

Ethylene oxide treatment of diets — The packaged diets were treated in glass dessicators attached through a manifold to a Cenco-Megavac pump. A mercury manometer served to measure the extent of evacuation in the dessicators and the pressure of the ethylene oxide–carbon dioxide (20:80 by weight) gas mixture (Liquid Carbonic) in the dessicators. The dessicators were first evacuated to a pressure of 30 mm of mercury, then the gas mixture was admitted to atmospheric pressure. At the end of the required treatment time, the residual gas mixture was removed by evacuation. To ensure removal of any remaining traces of the gas mixture, the dessicators and their contents were twice washed with room air which was filtered through a 30 × 2.5-cm cotton wool filter. Feed samples used for the bioassay were treated in 18-oz plastic "Whirl-Pak" bags, which contained up to 80-g feed. After gas treatment, the bags were sealed and stored at 4 C until used. The feed samples used for bacteriological examination were contained in capped test tubes and contained 1-, 5-, or 10-g feed. All treatments were carried out at room temperature.

Bacteriological procedures — Quantitative sterilization tests — Standard bacteriological sampling techniques were followed in the procurement of samples (American Public Health Association 1965). The number of viable bacteria present in the fish diets before and after treatment with ethylene oxide was determined using the procedures outlined in Standard Methods for the Examination of Water and Wastewater (American Public Health Association 1965). Samples (1, 5, or 10 g) were transferred aseptically to dilution bottles containing sufficient sterile buffered distilled water to give a 10^{-1} (w/v) dilution of each feed. The bottle was then shaken vigorously 25 times and duplicate serial dilutions were prepared in buffered distilled water. The viable mesophilic bacteria present were enumerated by the drop plate method of Miles and Misra (1938). Standard Methods agar (Baltimore Biological Laboratories) plates were incubated aerobically at 30 C for 48 hr, and anaerobically for 72 hr. Anaerobiosis was obtained using BBL Anaerobic Jars with Disposable Hydrogen–Carbon Dioxide Generator envelopes (BBL).

Qualitative sterilization tests — To test for sterility of feeds after ethylene oxide treatment, 10 ml of Trypticase Soy broth was added to each of 20 tubes containing 1 g ethylene oxide treated feed, 10 tubes were incubated aerobically, and 10 tubes were incubated anaerobically at 30 C for 5 days. Tubes were examined organoleptically for growth and were also streaked on plates of Trypticase Soy agar. Control tubes containing feed samples were subjected to evacuation and air washing but not to treatment with ethylene oxide.

The effect of ethylene oxide treatment on the viability of known bacterial species added to feeds was also tested. The indicator organisms used were *Aeromonas salmonicida* ATCC 14174, *Clostridium perfringens* ATCC 3624, *Escherichia coli* ATCC 11775, *Pseudomonas aeruginosa* ATCC 15442, *P. fluorescens* ATCC 13525, *Salmonella montevideo* (strain isolated from Diet 3, Trust 1971), *Staphylococcus aureus* ATCC 6538, and *Streptococcus faecalis* ATCC 8043. Different cultural conditions were required to prepare inocula of these test species. Cultures of *E. coli*, *P. aeruginosa*, *S. montevideo*, *S. aureus*, and *S. faecalis* were incubated at 37 C for 24 hr in Trypticase Soy broth, while *A. salmonicida* and *P. fluorescens* were incubated at 30 C for 24 hr in Brain Heart Infusion broth (BBL). *C. perfringens* was incubated anaerobically at 37 C for 48 hr in Trypticase Soy broth. The resulting cultures contained from 1×10^8 to 5×10^8 viable cells/ml. A *Bacillus subtilis* spore suspension which contained 9×10^8 viable spores/ml was prepared by the method of the AOAC (Association of Official Analytical Chemists 1970). A 0.1-ml aliquot of the inoculum culture was then added to 1-g samples of the feeds; the feed samples were allowed to dry by standing for 16 hr at room temperature and were then treated with ethylene oxide. After treatment, 5 ml of the appropriate broth was added aseptically to each sample and the tubes were incubated using the conditions used for inoculum growth. After 48 hr, tubes were subcultured to selective and indicator media. Viability of *A. salmonicida* was detected by growth and brown-pigment formation on Furunculosis agar (Difco), viability of *C. perfringens* was demonstrated by stormy clot formation in litmus milk, and *E. coli* was detected by lactose fermentation on MacConkey agar. Viability of *P. aeruginosa* was detected by growth and pyocyanin production on Pseudosel agar (BBL), *P. fluorescens* by growth and fluorescein production on Pseudomonas F agar (BBL), and *S. montevideo* was grown on Salmonella–Shigella agar (BBL), Vogel and Johnson agar (BBL) was used to demonstrate viability of *S. aureus* and Ethyl Violet Azide agar (BBL) was used to demonstrate viability of *S. faecalis*. Control inoculated feed samples were subjected to evacuation and air washing but were not treated with ethylene oxide.

Qualitative evaluation of the suitability of ethylene oxide sterilized feeds as bacterial media — The absence of significant carry-over concentrations of ethylene oxide in the feeds after treatment was tested bacteriologically. To each 1 g of ethylene oxide treated feed was added a 5-ml aliquot of sterile distilled water. Tubes were incubated at 30 C for 5 days and observed organoleptically for bacterial growth, and by streaking on plates of Trypticase Soy agar. The tubes were then inoculated with a 0.025-ml aliquot of the bacterial indicator organisms prepared as before. The tubes were then incubated as above and, after 48 hr, examined for bacterial growth.

Bioassay of ethylene oxide treated diets — The effects of ethylene oxide treatment of the diets on the growth rate of fishes were evaluated using juvenile rainbow trout (*S. gairdneri*). Trout eggs were obtained from spawning females in Lake Penask, B.C., on June 28, 1971[4]. On arrival at the laboratory, the eggs were placed in a

[4]Eggs were supplied by the B.C. Department of Recreation and Conservation.

vertical flow hatchery supplied with 2–3.5 liters/min of well water at 11–13 C. Complete details of the hatchery conditions and of the bacterial flora of these eggs has already been published (Trust 1972). Hatching was completed by July 31, 1971. The alevins were reared at 11–13 C using Diet 3 until November 10, 1971. These fish were used for the three growth rate experiments. The tanks used for this work were of porcelain coated metal 50 × 50 × 23 cm and contained 40 liters of well water. Each tank was supplied with 500 ml of well water/min.

Experiment 1 — Four representative groups of 100 fish were distributed into four tanks and fed Diet 1, which was treated with ethylene oxide, as follows: Tank 1, not treated (control group); Tank 2, treated 6 hr; Tank 3, treated 12 hr; and Tank 4, treated 24 hr. After 59 days the fish were weighed.

Experiment 2 — The fish used in Experiment 1 were pooled and distributed into eight tanks (50 fish/tank) and fed diets treated with ethylene oxide, as follows: Tank 1, Diet 1, not treated (control group); Tank 2, Diet 1, treated 6 hr; Tank 3, Diet 1, treated 12 hr; Tank 4, Diet 1, treated 24 hr; Tank 5, Diet 2, not treated (control group); Tank 6, Diet 2, treated 24 hr; Tank 7, Diet 3, not treated (control group); and Tank 8, Diet 3, treated 24 hr.

The fish were weighed after 26 and 53 days, when the experiment was terminated.

Experiment 3 — A separate group of 100 fish were fed on untreated Diet 3 for 59 days and were then distributed into two groups of 50 fish and fed Diet 3 treated with ethylene oxide, as follows: Tank 1, not treated (control group); and Tank 2, treated 24 hr.

The fish were weighed after a further 26 and 53 days, when the experiment was terminated.

The tanks used in all experiments were cleaned weekly with a detergent containing hypochlorite. The fish were fed to appetite at least three times daily, and were weighed by difference in water.

Results — The fish diets examined in this study contained 10^3–10^4 aerobic bacteria and 10^2–10^3 anaerobic bacteria/g. The numbers of aerobic bacteria in these fish diets were comparable to the numbers found in the other animal feeds examined (Table 1). Many of the constituents in the formulated feedstuffs contribute to the bacterial burden of the feed. This was shown with Diet 1. The constituents of Diet 1 were examined for their bacterial burden and the estimated number of organisms detected per gram of diet constituent were as shown in Table 1.

Treatment of the diets with ethylene oxide gas mixture rapidly reduced the bacterial counts in the pelleted feeds. The decrease in the number of viable aerobic bacteria when feeds were treated is shown in Table 2.

The qualitative tests for absolute sterility showed that the 3 diets were sterile after exposure to the sterilizing gas mixture for 12 hr. No growth was shown after incubation of gas treated diets to which broth had been added. When ethylene oxide sterilized feed was suspended in 5 ml of sterilized water, it supported normal growth of all of the test organisms.

TABLE 1. Effect of ethylene oxide treatment on the viability of aerobic bacteria in feedstuffs.

Diet tested	Viable aerobic cells/g of feedstuff after treatment with an ethylene oxide gas mixture			
	0 hr (*exposure*)	1 hr (*exposure*)	3 hr (*exposure*)	6 hr (*exposure*)
Diet 1	8.6×10^3	3.0×10^3	1.2×10^3	NCO[a]
Diet 2	5.4×10^3	7.2×10^2	NCO	NCO
Diet 3	2.0×10^3	1.3×10^3	NCO	NCO
Bran	2.6×10^4	NCO	NCO	NCO
Turkey grower	4.4×10^4	6.0×10^2	NCO	NCO
Duck grower	1.2×10^4	2.0×10^2	NCO	NCO
Chick starter	5.9×10^5	NCO	NCO	NCO

[a]NCO = No Countable Organisms.

TABLE 2. Bacterial burden of constituents of Diet 1.

Diet constituent	Aerobic bacteria/g	Anaerobic bacteria/g
Soybean	1.5×10^5	2.5×10^2
Corn	1.0×10^6	1.5×10^5
Wheat	1.3×10^5	1.5×10^2
Skim milk	3.6×10^2	2.4×10^2
Meat meal	9.0×10^3	8.8×10^2
Fish meal	2.3×10^3	2.8×10^2
Combined constituents	3.8×10^5	3.4×10^4
Combined constituents after grinding	1.3×10^5	3.3×10^3
Combined, ground, and pelleted diet	4.5×10^4	3.6×10^2

The results in Table 3 show that ethylene oxide treatment of dried pelleted fish feeds, containing 10^7 viable cells/g of feed, for 3 hr, killed *E. coli*, *P. aeruginosa*, and *P. fluorescens*. Treatment for 6 hr killed *A. salmonicida* and *S. montevideo*, a 12 hr treatment killed *C. perfringens* and *S. faecalis*, and a 21-hr exposure killed *S. aureus*. All these test species grew in the controls, which were not treated with the ethylene oxide, but which had been subjected to evacuation and to air washing.

The sporicidal activity of the ethylene oxide is shown in Table 4. A 1-hr exposure to the sterilizing gas mixture killed 40% of the *B. subtilis* spores in Diet 1, 7% of the spores in Diet 2, and 20% of the spores in Diet 3. Exposure for 6 hr killed 99% of the spores in Diet 1, 91% of the spores in Diet 2, and

TABLE 3. Effect of exposure to an ethylene oxide gas mixture on the viability of bacterial species in pelleted fish diets.[a]

Species	Growth of test species after ethylene treatment															
	Diet 1					Diet 2						Diet 3				
	0 hr	1 hr	3 hr	6 hr	12 hr	0 hr	1 hr	3 hr	6 hr	12 hr	24 hr	0 hr	1 hr	3 hr	6 hr	12 hr
Aeromonas salmonicida 14174	+[b]	+	+	0[c]	0	+	+	0	0	0	0	+	+	0	0	0
Clostridium perfringens 3624	+	+	+	+	0	+	+	+	0	0	0	+	+	+	0	0
Escherichia coli 11775	+	+	0	0	0	+	+	0	0	0	0	+	0	0	0	0
Pseudomonas aeruginosa 15442	+	+	0	0	0	+	+	0	0	0	0	+	+	0	0	0
Pseudomonas fluorescens 13525	+	+	0	0	0	+	+	0	0	0	0	+	+	0	0	0
Salmonella montivideo	+	+	+	0	0	+	+	+	0	0	0	+	+	+	0	0
Staphylococcus aureus 6538	+	+	+	0	0	+	+	+	+	+	0	+	+	+	+	0
Streptococcus faecalis 8043	+	+	+	0	0	+	+	+	+	0	0	+	+	+	0	0

[a]Feeds contained approximately 10^7 viable cells of each test species per gram of feed.
[b]+ indicates growth.
[c]0 indicates no growth.

TABLE 4. Effect of exposure to an ethylene oxide gas mixture on the viability of *Bacillus subtilis* spores in pelleted fish diets.

Diet tested	Viable cells/g of feedstuff after ethylene oxide treatment						
	0 hr (*exposure*)	1 hr (*exposure*)	3 hr (*exposure*)	6 hr (*exposure*)	12 hr (*exposure*)	21 hr (*exposure*)	30 hr (*exposure*)
Diet 1	1.0×10^7	6.0×10^6	9.8×10^5	1.1×10^5	NCO[a]	NCO	NCO
Diet 2	1.5×10^7	1.4×10^7	7.1×10^6	1.3×10^6	4.7×10^4	3.0×10^4	NCO
Diet 3	1.5×10^7	1.2×10^7	4.7×10^6	8.2×10^5	1.2×10^4	1.0×10^4	NCO

[a]NCO = No Countable Organisms.

95% of the spores in Diet 3. There were no countable spores in Diet 1 after 12-hr exposure. A 12-hr gas exposure killed more than 99% of the spores in Diets 2 and 3, and no countable spores were present after a 30-hr exposure. The control feeds, which were subjected to evacuation without treatment with the gas mixture, did not show a significant decrease in the number of viable spores present.

The three bioassay experiments revealed that ethylene oxide treatment of pelleted diets for up to 24 hr apparently did not adversely affect the growth of the test fish during the assay period. The results in the three bioassays are shown in Tables 5, 6, and 7. No mortality was shown in the three bioassays; however, seven fish in Experiment 2 were not growing well at the termination of the experiment. Two of these fish were being fed unsterilized Diet 2, and five of the fish were being fed with sterilized Diet 2.

Discussion — The present study has confirmed earlier work on the efficiency of ethylene oxide as a satisfactory agent (when used under the correct conditions) to kill bacterial spores and vegetative cells in dry solid laboratory animal diets. Kelsey (1967) has provided an excellent critical review of the conditions required for the proper use of ethylene oxide. There is a substantial reduction in the number of viable bacteria comprising the microbial flora of feeds after exposure to ethylene oxide for 1–3 hr. To achieve a greater kill requires longer exposure, and a 6–12-hr exposure was sufficient to sterilize feedstuffs with a count of 10^3–10^5 viable cells/g.

TABLE 5. Effect of exposure of Diet 1 to an ethylene oxide gas mixture on the growth of rainbow trout (*Salmo gairdneri*).

Days of feeding	Mean weight[a]/Fish (*g*)			
	0 hr exposure	6 hr exposure	12 hr exposure	24 hr exposure
0	1.0	1.1	1.1	1.1
59	1.3	1.5	1.5	1.5

[a]Total of 100 fish in experiment.

TABLE 6. Effect of exposure of Diets 1, 2, and 3 to an ethylene oxide gas mixture on the growth of rainbow trout (*Salmo gairdneri*).

Exposure time (*hr*)	Mean weight[a]/Fish (*g*)		
	0 days feeding	26 days feeding	53 days feeding
Diet 1			
0	1.7	1.9	2.7
6	1.8	2.4	2.9
12	1.9	2.2	2.7
24	1.8	2.3	2.7
Diet 2			
0	1.7	2.1	3.6
24	1.7	2.1	3.6
Diet 3			
0	1.8	2.7	4.6
24	1.8	2.3	4.6

[a]Total of 50 fish in experiment.

TABLE 7. Effect of exposure of Diet 3 to an ethylene oxide gas mixture on the growth of rainbow trout (*Salmo gairdneri*).

Days of feeding	Mean weight[a]/Fish (*g*)	
	0 hr exposure	24 hr exposure
0	3.8	3.8
26	5.4	5.8
53	8.2	9.3

[a]Total of 50 fish in experiment.

Feeds to which 10^7 vegetative cells/g had been added required a 12–21-hr exposure, and the feeds to which 10^7 *B. subtilis* spores had been added required an exposure of longer than 21 hr to achieve sterility.

Earlier studies (Trust 1971; Trust and Money 1972) have shown that fish diets can be a source of bacterial species that are pathogenic to man and fish. The present study suggests that gaseous sterilization of feeds is effective in killing such organisms. It is also apparent that this technique destroys those bacteria commonly used as indicator organisms for faecal pollution of water (*E. coli*, *C. perfringens*, *S. faecalis* — American Public Health Association 1965). Use of sterilized feeds should reduce the numbers of these species in the effluent water of hatcheries. The destruction of these organisms and pathogenic species such as *Salmonella* in the feeds and the hatchery effluents would help eliminate any hazards presented to human and animal populations downstream from the hatchery. Also, since fish have been implicated as vectors of bacterial diseases of man (Janssen 1971), it is important that such bacteria be eliminated from the diets of fish being raised for human consumption. Since ethylene oxide is considered effective against all types of microorganisms including fungal cells, fungal spores, rickettsia, and viruses (Hoffman 1971), the gaseous sterilization treatment would also lessen the problems of transmission of these agents in feeds.

From the practical standpoint, ethylene oxide treatment of fish diets would reduce the possibility of bacteriological damage to feed quality and reduce the need to monitor the bacteriological quality of fish diets. The use of sterile feeds would also aid in the maintenance of sanitary conditions in hatcheries by eliminating a source of bacterial contamination. The equipment used to sterilize the feeds could also be used to sterilize other articles such as nets and workers' clothing.

Various sterile solid diets that satisfy the dietary requirements of other laboratory animals have been developed (Reddy et al. 1968). The results obtained in this initial study indicate that the fish diets can also be effectively sterilized without significant damage to the feed, and with no harmful nutritional effects being shown by the fish tested. The growth rates of the fish fed with sterilized feed were equal to or greater than those of the fish fed unsterilized feed. This was true for fish growing on three different diets and for fish growing at three different growth rates. These findings confirm the findings of others with rats, mice, and guinea pigs (Charles et al. 1965). Further long-term growth studies are currently in progress to evaluate the effects of ethylene oxide treatment of feeds on the growth of fish. The successful application of this apparently simple technique to provide adequate sterile diets will facilitate the raising of axenic fish in adequate numbers. Such fish should provide an ideal tool in the understanding of the role of the fish microflora in fish growth, and will be useful in fundamental studies on the immunology and nutrition of fishes.

AMERICAN PUBLIC HEALTH ASSOCIATION. 1965. Standard methods for the examination of water and wastewater. 12th ed. APHA, Inc., New York, N.Y. p. 567–626.

ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS. 1970. Official methods of analysis of the Association of Official Analytical Chemists. 11th ed. AOAC, Washington, D.C. 754 p.

CHARLES, R. T., D. E. STEVENSON, AND A. I. T. WALKER. 1965. The sterilisation of laboratory animal diet by ethylene oxide. Lab. Anim. Care 15: 321–324.

COATES, M. E. 1970. The sterilization of laboratory animal diets, p. 38–48. *In* W. D. Tavernor [ed.] Nutrition and disease in experimental animals. Baillière, Tindall and Cassell Ltd., London.

HOFFMAN, R. K. 1970. Toxic gases, p. 225–258. *In* W. D. Hugo [ed.] Inhibition and destruction of the microbial cell. Academic Press, London.

JANSSEN, W. A. 1970. Fish as potential vectors of human bacterial diseases. *In* S. F. Snieszko [ed.] A symposium on diseases of fishes and shellfishes. Spec. Publ. 5: 284–290. American Fisheries Society, Washington, D.C.

KELSEY, J. C. 1967. Use of gaseous antimicrobial agents with special reference to ethylene oxide. J. Appl. Bacteriol. 30: 92–100.

KERELUK, K., AND R. S. LLOYD. 1969. Ethylene oxide sterilization: a current review of principles and practices. J. Hosp. Res. 7: 8–75.

MILES, A. A., AND S. S. MISRA. 1938. The estimation of the bactericidal power of the blood. J. Hyg. 38: 732–749.

REDDY, B. S., B. S. WOSTMANN, AND J. R. PLEASANTS. 1968. Nutritionally adequate diets for germ-free animals, p. 87–111. *In* M. E. Coates [ed.] The germ-free animal in research. Academic Press, Inc. London.

SYKES, G. 1967. Disinfection and sterilization. 2nd ed. E. and F. N. Spon Ltd. London. p. 202–225.

THATCHER, F. S., AND D. S. CLARK. 1968. Microorganisms in food: their significance and methods of enumeration. University of Toronto Press, Toronto. p. 3–31.

TRUST, T. J. 1971. Bacterial counts of commercial fish diets. J. Fish. Res. Board Can. 28: 1185–1189.

TRUST, T. J. 1972. The bacterial population in vertical flow tray hatcheries during incubation of salmonid eggs. J. Fish. Res. Board Can. 29: 567–571.

TRUST, T. J., AND V. G. MONEY. 1972. Bacterial population of diets for aquarium fishes. J. Fish. Res. Board Can. 29: 429–433.

WOOD, A. J. 1941. The value of certain gases for the sterilization of fresh and salt fish. Annual Reports of the Atlantic Fisheries Experimental Station. The Fisheries Research Board of Canada. Section III. A47–A52.

Arsenic, Cadmium, Copper, Mercury, and Zinc in Some Species of North Atlantic Finfish

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Arsenic, cadmium, copper, mercury, and zinc analyses of 91 individuals representing 35 species of North Atlantic finfish (Chondrichthys and Osteichthys) indicate that these metals occur at similar levels in both inshore and offshore species. Chondrichthys and Osteichthys have similar concentrations of all the metals, excepting arsenic which is higher in Chondrichthys. Analyses of various tissues in Chondrichthys reveal higher metal concentrations in the liver except for mercury which was higher in muscles.

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Des analyses effectuées sur 91 individus représentant 35 espèces de poissons (chondrichthyens et ostéichthyens) de l'Atlantique Nord démontrent que l'arsénic, le cadmium, le cuivre, le mercure, et le zinc sont présents en concentrations semblables chez les espèces du littoral et chez celles de la haute mer. Tous les métaux sont présents en concentrations semblables chez les chondrichthyens et les ostéichthyens, sauf l'arsenic, qui est plus élevé chez les chondrichthyens. L'analyse de divers tissus des chondrichthyens révèle des concentrations de métaux plus élevées dans le foie, à l'exception du mercure, qui est plus élevé dans les muscles.

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IN recent years, the concentration of heavy metals in marine fishes has received much interest. This interest has been directed towards the commercial and sports fishes since high levels of heavy metals in these species represent a potential human health hazard. Other, commercially unimportant, finfish species have received little attention. It is important, however, that more information on these species be obtained to understand whether high levels of metals in some fishes, such as tuna and swordfish, are due to generally increased levels of the metals in the marine ecosystem or are a physiological peculiarity of the particular species. It is also important to have a broad understanding of metal levels in a large variety of finfish to establish a baseline for comparison with possible future changes.

This report is the result of a study of heavy metal concentrations in the marine biota of the North Atlantic Ocean and was a part of an International Decade of Ocean Exploration baseline study (for summary see Anon. 1972) of the quality of the marine environment. During the study, 91 individuals representing 35 species of North Atlantic finfish were analyzed for arsenic, cadmium, copper, mercury, and zinc. The samples included members of Osteichthys and Chondrichthys with offshore and inshore species of both represented. Various tissues of Chondrichthys were also analyzed for the metals.

Methods — Offshore fishes were collected in various locations east of the Gulf Stream in the vicinity of the Sargasso Sea. All inshore species were collected along the coasts of South Carolina, Georgia, and Florida. All samples were stored frozen in plastic bags prior to analysis.

Muscle samples were collected from tissues just below the dorsal fin in the smaller fishes. In the larger fishes several samples of muscle were taken from various locations and each was analyzed separately. The results were pooled to obtain the single value reported. The whole body minus the head was used in the analysis of myctophids and anchovies.

Samples for arsenic, cadmium, copper, and zinc analysis were wet ashed using fuming nitric acid at 60 C, dissolved in concentrated nitric acid, and brought to volume with distilled water. After each sample had been analyzed for sodium, calcium, magnesium, and iron to correct for matrix effects, they were analyzed by flame atomic absorption using calibration standards made up in a similar matrix. For arsenic, the procedure for analysis differed somewhat after wet ashing. After dissolution, the samples were freed of nitric acid by purging with

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CO_2. Samples were then diluted with a solution containing 20% HCl and 5% H_2SO_4, followed by treatment with potassium iodide and stannous chloride to reduce the arsenic from As^{+5} to As^{+3}. Arsine gas was then liberated by the addition of zinc to the acid solution contained in a reaction flask. The flask was purged with argon through a hydrogen sulfide trap into the hydrogen argon flame of the atomic absorption system. This technique greatly diminishes the interference encountered in the UV range with conventional flame atomic absorption techniques, and is similar to that described by Dalton and Malonski (1971).

Tissue samples for mercury analyses were wet digested in concentrated sulfuric and nitric acids on a water bath at 60 C overnight. After digestion the samples were diluted and further oxidized with potassium permanganate

TABLE 1. Mean and standard deviation of replicate analyses of metals in NBS Standards. Values expressed as μg/g dry weight.

	As	Cd	Cu	Hg	Zn
Orchard Leaves					
Present Study	12.8±1.5	0.3±0.1	11±1	0.15±0.03	27±2
NBS Value	14±2	0.11±0.02	12±1	0.155±0.015	25±2
Bovine Liver					
Present Study	<1.0	0.2±0.2	120±12	0.012±0.005	80±5
NBS Value	0.06	0.27±0.04	193±10	0.016±0.002	130±10

TABLE 2. Arsenic, cadmium, copper, mercury, and zinc in muscle tissue of Osteichthys from inshore (I) and offshore (O) locations.

Family	Species	Habitat	No. of individuals analyzed	μg/g (Dry weight)				
				As	Cd	Cu	Hg	Zn
Arridae	*Bagre marinus*	I	1	<1.0	<0.1	0.7	3.0	12
Anguillidae	*Anguilla rostrata*	I	1	<1.0	<0.1	0.8	2.7	25
Bothidae	*Paralichthys lethostigma*	I	3	<1.0	1.3	3.0	–	39
Carangidae	*Decapterus punctatus*	O	8	1.8	<0.1	2.0	0.4	18
Congridae	*Conger* sp.	I	1	<1.0	0.3	8.7	1.2	41
Engraulidae[a]	*Anchoa mitchelli*	I	6	2.1	0.6	10.0	–	397
Mugilidae	*Mugil cephalus*	I	11	<1.0	<1.0	1.9	0.1	17
Myctophidae[a]	*Hygophum hygomi*	O	2	<1.0	<0.1	3.4	0.3	15
	Ceratoscopelu warmingil	O	2	<1.0	0.7	2.2	0.2	35
	Notoscopelus caudispinous	O	2	<1.0	0.4	3.2	0.2	81
	Lobianchia dofleini	O	1	<1.0	1.6	23.0	0.2	49
	Lepidophanes indicus	O	1	<1.0	0.9	13.0	0.2	56
	Diaphus mollis	O	1	<1.0	0.8	7.0	0.1	34
	Lampanyctus pusillus	O	1	<1.0	1.6	23.0	0.3	48
	Lampanyctus pusillus	O	2	<1.0	0.4	2.7	0.2	27
Ophichthidae	*Ophichthus ocellatus*	I	2	<1.0	0.3	2.7	1.3	26
	Ophichthus gomesi	I	2	<1.0	<0.1	1.5	0.6	21
Pericichthyidae[b]	*Morone saxatilis*	I	1	1.8	<0.1	2.5	4.5	12
Pomatomidae	*Pomatomis saltatrix*	I	1	–	–	–	0.9	–
Sciaenidae	*Bairdiella chrysura*	I	5	–	0.4	3.5	–	30
	Cynoscion nebulosus	I	5	2.5	0.5	9.5	1.5	21
	Leiostomus xanthurus	I	5	–	0.5	8.4	–	20
Scombridae	*Euthynnus alletteratus*	O	5	1.1	0.2	1.6	1.4	8
	Scomberomorus maculatus	I	6	1.8	0.3	2.3	0.4	15
Serranidae	*Unidentified*	O	1	<1.0	0.2	2.5	2.3	11
	Centropristes striatus	I	1	6.4	0.4	<0.3	0.8	7

[a]Whole body analyzed minus head.
[b]Collected in Savannah River which contains relatively high industrial Hg.

and potassium disulfate, after which reduction of mercury to its zero valence state was accomplished with stannous sulfate. Samples were then aerated with a peristaltic pump into a cold vapor atomic absorption system for analysis, comparing the resulting absorption with standards.

All samples were analyzed in at least duplicate and reported on a dry weight basis. The percentage moisture varied from 65 to 78% on all samples analyzed. The precision of the analyses based on replicate samples at the levels encountered, expressed as standard deviations, was ± 10% for copper, mercury, and zinc, ± 20 to 30% for cadmium, and ± 15 to 10% for arsenic. An indication of the accuracy of these analyses can be obtained from Table 1 which compared our results on standards with those of the National Bureau of Standards.

Results and Discussion — The concentrations of all the metals analyzed in Osteichthys appear to

TABLE 3. Arsenic, cadmium, copper, mercury and zinc in tissues of Chondrichthys from inshore (I) and offshore (O) locations.

Family	Species	Habitat	No. of individuals analyzed		(μg/g Dry Weight) As	Cd	Cu	Hg	Zn
Carcharinidae	*Carcharhinus falciformis*	O	2	Muscle	<1.0	1.0	2.1	5.3	10
				Liver	20	5.0	4.9	1.6	19
				Kidney		2.6	5.7	3.3	25
				Brain		<0.2	8.4	2.0	10
				Gonads		<0.2	<1.0	9.0	10
				Gills		<0.2	6.3	2.2	24
				Spleen	3.3	<0.2	4.6		28
	Carcharhinus milberti	I	1	Muscle				1.0	
				Liver	11.2	<0.1	2.7	0.4	9
	Carcharhinus obscurus	I	1	Muscle	6.0	2.1	1.5	4.2	19
				Liver	10	1.6	1.3	3.3	16
				Brain	10	<0.1	8.4	2.9	27
				Pup (total)	2.2	0.2	1.6	1.4	12
				Pup (total)	2.8	0.3	2.4	0.5	14
Myliobatidae	*Rhinoptera bonusus*	I	3	Muscle	4.7	0.2	2.3	1.0	8
				Liver	17	0.6	13	0.8	9
				Brain	5.4	<0.1	10	0.3	36
				Stomach	3.7	0.4	7.0	0.4	34
				Spiral	2.2	0.5	5.2	0.2	29
				Spleen	3.8		3.6	0.4	32
				Uterus	4.9	0.6	3.4	0.1	30
Rajidae	*Raja eglanteria*	I	1	Muscle	19	0.6	3.2	1.3	20
				Liver	6.0	<0.2	44	0.5	44
				Yolk sac	22	0.5	4.4	0.2	31
Rhinobatidae	*Rhinobatis lentiginous*	I	2	Muscle	11	0.4	2.2	1.2	11
				Liver	16	1.4	6.6	0.4	29
				Stomach	15	0.4	6.2	0.5	38
				Yolk sac	1.6	0.2	2.7	0.1	19
Sphyrnidae	*Sphyrna tiburo*	I	1	Muscle	14	0.4	3.0	2.3	8
				Liver	17	0.9	3.6	0.4	13
				Stomach	8.9	0.9	9.2	0.6	28
				Spleen	17	0.6	2.4	0.4	25
				Ovary	17	0.7	2.4	0.7	36
	Sphyrna lewini	I	1	Muscle	2.2	<0.1	2.0	2.7	15
				Liver	6.0	<0.1	6.2	0.4	16
				Stomach	1.8	<0.1	10	0.9	40
				Intestine	1.9	0.8	5.7	0.4	
Squalidae	*Squalus acanthius*	I	2	Muscle	10	0.4	2.3	2.7	12
				Liver	5.7	1.0	4.5	0.4	15
				Stomach	<1.0	3.7	4.8	1.4	70
				Spleen	9.8	1.4	16	1.8	30
				Yolk sac	9.1	0.2	0.9	0.4	16
				Embryo	2.6	0.7	3.0	0.2	37

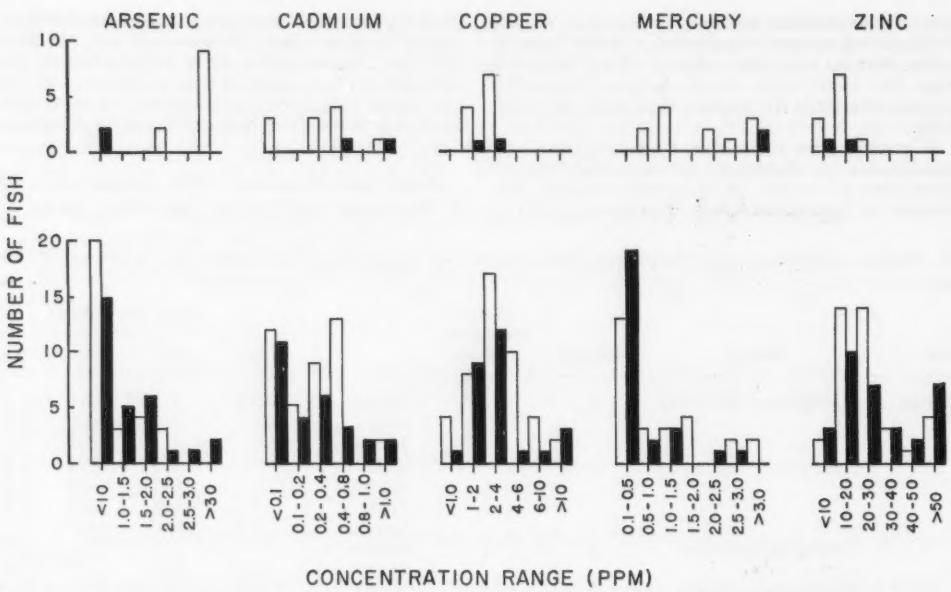

FIG. 1. Metal distribution in fish. (Upper and lower histograms represent distribution for Chondrichthys and Osteichthys, respectively. Black and white bars represent offshore and inshore species, respectively. Concentration is on dry weight basis.)

vary widely (Table 2). Most of these fish contained arsenic concentrations below the sensitivity of analysis; however, some contained considerably higher concentrations up to 6.4 ppm. Mercury was also generally low, however, in some individuals concentrations of a few ppm were observed. In all fish studied, cadmium, copper, and zinc concentrations appear similar with the exception of the smaller planktivorous fishes such as anchovies and myctophids. These similar and lower trophic level fishes have much higher concentrations of zinc, copper, and cadmium than the other fishes. This suggests a depletion in these metals up the food chain, since plankton has even higher concentrations of these metals (Windom et al. 1972; Riley and Roth 1971).

In general, the copper and zinc in Chondrichthys is similar to that found in Osteichthys (Table 3). Based on the few Chondrichthys samples analyzed, mercury and cadmium in their muscle tissues tend toward higher concentrations than Osteichthys. Arsenic, however, appears to be definitely higher in Chondrichthys. Most tissues of Chondrichthys have concentrations of the metals similar to the muscle except for the liver, which has higher concentration of arsenic, cadmium, copper, and zinc. None of the reproductive organs, embryos, or pups of Chondrichthys appear to concentrate, to any significant degree, any of the metals with the possible exception of slightly elevated levels of zinc.

Figure 1 shows histograms of the frequency of fishes having concentrations of the various metals at different levels. It is clear from this figure that in general there is no tendency toward any difference between offshore and inshore species, with the possible exception, on the basis of the limited number of samples, of arsenic concentrations in inshore Chondrichthys. The overlapping range in the concentrations of the metals between the Chondrichthys and the Osteichthys is clearly apparent in Fig. 1 and is similar to those reported by Vinogradov (1953) for marine fishes and Uthe and Bligh (1971) for freshwater fishes.

The above limited data suggest that there is little difference in metal concentrations between offshore and inshore species of finfish in the North Atlantic. Of the variations seen between different species, most appear to be related to size and, hence, are probably physiologically controlled.

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ANON. 1972. Baseline studies of pollutants in the marine environment and research recommendation. IDOE Baseline Conf. May 24–26, New York, N.Y. 54 p.

DALTON, E. F., AND A. J. MALONSKI. 1971. Note on the determination of arsenic by atomic absorption by arsine generation into an argon-hydrogen entrained air flame. At. Absorpt. Newsl. 10: 92–93.

RILEY, J. P., AND I. ROTH. 1971. The distribution of trace elements in some species of phytoplankton grown in culture. J. Mar. Biol. Ass. U.K. 51: 63–72.

UTHE, J. F., AND E. G. BLIGH. 1971. Preliminary survey of heavy metal contamination of Canadian freshwater fish. J. Fish. Res. Board Can. 28: 786–788.

VINOGRADOV, A. P. 1953. The elementary chemical composition of marine organisms. Sears Found. Mar. Res. 2.

WINDOM, H., F. TAYLOR, AND R. STICKNEY. 1972. Mercury in North Atlantic plankton. J. Cons. Cons. Perma. Int. Explor. Mer. (In press)

Response of Lobsters *Homarus americanus* to Odor Solution in the Presence of Bleached Kraft Mill Effluent

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McLEESE, D. W. 1973. Response of lobsters *Homarus americanus* to odor solution in the presence of bleached kraft mill effluent. J. Fish. Res. Board Can. 30: 279–282.

The feeding response of lobsters (*Homarus americanus*) is not affected by bleached kraft mill effluent (BKME) at low concentrations.

In test runways, lobsters walked upstream toward the source of dilute solutions of freeze-dried cod muscle extract (FDC). Differences between test and control responses occurred in only two of 12 comparisons when FDC was associated with 10, 50, and 100% BKME (Series I and II). With six concentrations of BKME, ranging from 0.01 to 2.0%, maintained in the runways (Series IV), responses to FDC were variable, but average responses for controls and tests did not differ. Conditions in the latter tests most closely approximated those in nature where some lobsters could be exposed continuously to low concentrations of BKME.

McLEESE, D. W. 1973. Response of lobsters *Homarus americanus* to odor solution in the presence of bleached kraft mill effluent. J. Fish. Res. Board Can. 30: 279–282.

Le comportement alimentaire du homard (*Homarus americanus*) n'est pas affecté par l'effluent blanchi d'usine de papier kraft (BKME) à faibles concentrations.

Sur les rampes d'essais, les homards marchent à contre-courant vers la source de solutions diluées d'extrait lyophilisée de muscle de morue (FDC). Lorsque BKME à des concentrations de 10, 50 et 100% (séries I et II) est ajouté à FDC, la réponse des homards ne diffère de celle des témoins que dans deux des 12 comparaisons. A six concentrations de BKME allant de 0.01 à 2.0% (série IV), la réponse à FDC est variable, mais la moyenne ne diffère pas entre les témoins et les sujets d'essais. Cette dernière série d'essais se rapproche le plus des conditions qui existent dans la nature, où des homards peuvent être exposés de façon continue à de basses teneurs de BKME.

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THIS study was undertaken to determine if bleached kraft mill effluent in sea water affects the ability of lobsters (*Homarus americanus*) to detect and respond to odor.

A bleached kraft mill near Pictou, Nova Scotia, began production in September 1967. Effluent from the mill discharges into a retention pond from which, after about 40 days, it flows through a diffuser pipe to an estuary. In surveys, effluent was visible as a band varying in width from 75 to 250 m for a distance of 3 km alongshore east of the estuary mouth (Scarratt 1969a,b). BKME concentration on bottom underlying this area ranged from 0.05% in offshore positions to about 0.7% close inshore.

Lobster landings in Pictou County (Fishery Districts 11 and 12) have declined from 2.5 million lb in 1955 to about 1.0 million lb in 1967, and 0.8

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million lb in 1970 (Dominion Bureau of Statistics). Previous studies indicate that BKME probably does not have an immediate or direct effect on abundance and distribution of larval or adult lobsters (Sprague and McLeese 1968a,b; McLeese 1970a; Scarratt and Raine 1967; Scarratt 1968, 1969a).

Stimulation of chemoreceptors by food odors carried in solution by water currents initiates food search behavior in lobsters, and is involved in their attraction to baited traps (McLeese 1970b). Exposure to sublethal concentration of detergents has been shown to impair feeding behavior in certain fish (Bardach et al. 1965; Foster et al. 1966), and several synthetic surfactants reversibly block sensory discharge in the olfactory epithelium of Atlantic salmon, *Salmo salar* (Sutterlin et al. 1971). The possibility remains that BKME could affect the ability of lobsters to respond to odor either because of odor masking from interaction of odor and BKME, or, assuming that BKME might contain surfactants, because of reversible or irreversible blocking of the chemoreceptors.

Materials and methods — BKME was collected from the retention pond close to the spillway, brought to St. Andrews, N.B., the same day, and stored outdoors in tightly closed plastic containers.

The apparatus consisted of a pair of wooden troughs (2.1 × 1.2 m with a water depth of 10 cm), each divided into four runways (0.5 × 0.3 m). Sea water at 10 C from a constant level tank flowed through each of the eight runways at the rate of 9 liters/min. A mature intermoult lobster weighing about 450 g was placed in each runway. The lobsters were temporarily blinded with aluminum foil eyeshields (McLeese 1970b). The animals were not fed for 1 week prior to experiments. At the end of each day's testing, a 2-g piece of cod muscle was placed in the upstream end of each runway to reward the lobsters without satiating them. At the termination of each experimental series, the animals were replaced with others that had not been exposed to BKME.

Only those lobsters initially at rest between the downstream barrier and the halfway mark in the runway were observed. When they responded to dilute solutions of freeze-dried cod muscle extract (FDC), they became alert and walked upstream toward the odor source (McLeese 1970b). The measure of response reported is the percentage of animals reaching the upstream barrier within 3 min of release of test solution.

The FDC was prepared from the solution collected as frozen cubes of cod muscle were thawed. In preliminary tests, maximum responses occurred with test solutions of 1 and 10^{-1} g FDC/liter of sea water; responses decreased progressively at lower concentrations with no response at 10^{-5} g/liter and lower. Standard dilutions of 10^{-1} and 10^{-2} g/liter were used to determine if BKME affected response differently at odor concentrations that caused maximum and less than maximum response.

For each runway, 150 ml of odor solution was released from a funnel immediately under the upstream barrier at the rate of 50 ml/min. Dye released in the same quantity and time, to observe the diffusion and movement of material added to the runways, reached the downstream barrier in about 1 min. The dye stream was narrow near the upstream barrier, gradually widening to occupy the full width of the runway at the halfway mark. At 3 min, dye strength at the upstream barrier was about 5% of the initial concentration and averaged 0.5% between the halfway mark and the downstream barrier.

Three methods of adding BKME to the runways were used: In Series I, 150 ml of FDC solution in sea water was released to each runway at the rate of 50 ml/min and an equal volume of either 10 or 50% BKME, diluted with sea water or 100% BKME, was released at the same time and rate. Tests were done hourly from 900 to 1700 hr during 5 days with order of testing rotated to average out possible effects related to time of day or to order of presentation of FDC and BKME concentrations except that tests at 900, 1200, and 1500 hr were controls. Series II was similar to Series I except that the FDC was dissolved in 10, 50, or 100% BKME solutions about 10 min before a test. Lobsters could receive a variety of stimuli in Series I, including combinations of FDC and sea water, BKME and sea water, and FDC and BKME against a seawater background. In Series II, they would be exposed to a combination of FDC and BKME against a seawater background.

In Series III, FDC was dissolved in sea water and in distilled water to determine if low density of the BKME solutions, as in Series II, affected the distribution of the FDC thereby affecting the response.

In Series IV, uniform concentrations of BKME in the runways were obtained by continuously adding 100% BKME at appropriate rates to the seawater inflow to the header tank for 4 hr/day. Six concentrations ranging from 0.01 to 2.0% BKME were provided. Only one concentration was tested during a day, starting with the lowest and using progressively higher concentrations on succeeding days. Each day the animals were tested at hourly intervals; the first two tests were controls, the next four were with the appropriate BKME background concentration and the last two were controls. In this series the lobsters were exposed to FDC against uniform low concentrations of BKME in sea water.

The observed frequencies of response from each series were compared for statistical differences using the normal approximation (Brownlee 1960).

Results and discussion — In Series I and II there were only two significant differences in twelve comparisons of test and control responses (Table 1). In both cases, test responses were less than control responses, but there was no consistency with respect to series treatment, to FDC strength, or to BKME concentration. If there is a BKME effect, it is relatively minor. With the exception of the abnormally low response at 50% BKME in Series II, other responses at 50 and 100% BKME ranged from 60 to 89% of the control responses. There was no lessening of control responses following exposure

to the BKME solutions indicating that, if BKME did block the chemoreceptors, the effect was reversible.

It is unlikely that BKME had a chemical effect on the FDC because there was no significant change in response with FDC dissolved directly in 100% BKME (Series II).

In Series III, the responses with FDC dissolved in sea water and distilled water (Table 1) were not

TABLE 1. Response of lobsters to freeze-dried cod muscle extract added to the runways simultaneously with 10, 50, and 100% BKME (Series I), with FDC dissolved in 10, 50, and 100% BKME (Series II), and in distilled water (Series III).

Initial odor conc. (g FDC/liter)	Initial BKME conc. (%)	Estimated BKME conc. in runways: downstream half (%)	Estimated BKME conc. in runways: upstream barrier (%)	Series I: No. trials	Series I: Response (%)	Series II: No. trials	Series II: Response (%)
10^{-1}	0	0	0	55	55	26	65
	10	0.05	0.5	38	58	26	62
	50	0.25	2.5	39	49	25	57
	100	0.5	5.0	37	33[a]	22	41
10^{-2}	0	0	0	62	39	33	42
	10	0.05	0.5	39	44	23	35
	50	0.25	2.5	39	33	16	6[a]
	100	0.5	5.0	39	31	21	29
10^{-1} in sea water		0	0	32	63	Series III	
10^{-1} in distilled water		0	0	32	60		

[a]Significantly different from control and 10% BKME responses (P .05).

TABLE 2. Response of lobsters to freeze-dried cod muscle extract at two concentrations with background levels of 0.01 to 2.0% BKME in the test runways.

Initial odor conc. (g FDC/liter)	Controls: No. trials	Controls: Response (%)	BKME Background (%)	Tests: No. trials	Tests: Response (%)
10^{-1}	9	78	0.01	11	55
	9	56	0.05	8	50
	11	55	0.1	11	64
	9	45	0.5	10	80[a]
	9	56	1.0	9	67
	13	69	2.0	15	80
Totals	60	60		64	67
10^{-2}	8	25	0.01	10	50[a]
	11	45	0.05	14	50
	9	45	0.1	9	45
	11	64	0.5	9	33
	9	56	1.0	10	40
	13	46	2.0	13	46
Totals	61	48		65	45

[a]Significantly different from control response (P .05).

different, indicating that low density of FDC solutions does not affect the responses under these experimental conditions.

In Series IV, there was considerable variation in control responses at both FDC concentrations (Table 2). Because of these variations, caused partly by the small number of trials in each test, small differences between controls and their corresponding tests with BKME background cannot be detected. Comparison of the paired control and test data showed differences in only two of twelve pairs; in each case the test response was the greater. Also the average responses for the pooled controls and tests with BKME background did not differ. As in Series I and II, if there is a BKME effect, it is relatively minor with no indication that BKME eliminates the response.

The impression gained during surveys (Scarratt 1969a) is that BKME in nature occurs over a relatively small area, is at low concentration (0.05–0.7%) on the bottom, and is relatively uniform except at the boundary between clean and contaminated water. Consequently, any lobsters in affected areas would be exposed continuously to low concentrations of BKME. The possibility of odor solutions being exposed initially to high concentrations of BKME as in Series II seems remote, in part because of the coincident reduction in salinity. The estimated concentrations of 0.05 to 0.5% BKME in the downstream half of the runways in Series I and II, and background concentrations of 0.01 to 0.5% in Series IV, are within the range some lobsters in nature could encounter. The estimated levels of $2\frac{1}{2}$ and 5% near the upstream barrier, in 50 and 100% BKME tests in Series I and II and the background levels of 1 and 2% in Series IV, generally exceed the range. Conditions in Series IV most closely approximate conditions in nature, except that exposure to BKME was for 4 hr a day rather than continuous.

Regardless of method, the results indicate that exposure to low concentrations of BKME for short periods does not reduce the response of lobsters to FDC solutions or, if so, to a minor extent only. The possibility that long-term exposure might affect the response, or that other behavior stimulated by chemoreceptors may be affected, was not tested.

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BARDACH, J. E., M. FUJIYA, AND A. HOLL. 1965. Detergents: effects on the chemical senses of the fish *Ictalurus natalis* (Le Sueur). Science 148: 1605–1607.

BROWNLEE, K. A. 1960. Statistical theory and methodology in science and engineering. John Wiley & Sons Inc., New York, N.Y. 570 p.

FOSTER, N. R., A. SCHEIER, AND J. CAIRNS, JR. 1966. Effects of ABS on feeding behaviour of flagfish, *Jordanella floridae*. Trans. Amer. Fish. Soc. 95: 109–110.

MCLEESE, D. W. 1970a. Behaviour of lobsters exposed to bleached kraft mill effluent. J. Fish. Res. Board Can. 27: 731–736.

1970b. Detection of dissolved substances by the American lobster (*Homarus americanus*) and olfactory attraction between lobsters. J. Fish. Res. Board Can. 27: 1371–1378.

SCARRATT, D. J. 1968. Distribution of lobster larvae (*Homarus americanus*) off Pictou, Nova Scotia. J. Fish. Res. Board Can. 25: 427–430.

1969a. Lobster larvae off Pictou, Nova Scotia, not affected by bleached kraft mill effluent. J. Fish. Res. Board Can. 26: 1931–1934.

1969b. Bleached kraft mill effluent near Pictou, N. S., and its effect on the marine flora and fauna with a note on the Pictou Co. lobster landings. Fish. Res. Board Can. MS Rep. 1037: 21 p.

SCARRATT, D. J., AND G. E. RAINE. 1967. Avoidance of low salinity by newly hatched lobster larvae. J. Fish. Res. Board Can. 24: 1403–1406.

SPRAGUE, J. B., AND D. W. MCLEESE. 1968a. Toxicity of kraft pulp mill effluent for larval and adult lobsters, and juvenile salmon. Water Res. 2: 753–760.

1968b. Different toxic mechanisms in kraft pulp mill effluent for two aquatic animals. Water Res. 2: 761–765.

SUTTERLIN, A., N. SUTTERLIN, AND S. RAND. 1971. The influence of synthetic surfactants on the functional properties of the olfactory epithelium of Atlantic salmon. Fish. Res. Board Can. Tech. Rep. 287: 14 p.

A Stimulatory Apparatus for Studying the Olfactory Activity in Fishes

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An apparatus was designed for stimulating the olfactory organs of fishes. The advantage of this apparatus is that it can easily control the stimulus time and the interval between two stimuli either independently or sequentially. The apparatus can also, with slight modification, be applied to the gustatory systems.

HARA, T. J., Y. M. C. LAW, AND E. VAN DER VEEN. 1973. A stimulatory apparatus for studying the olfactory activity in fishes. J. Fish. Res. Board Can. 30: 283–285.

Nous avons mis au point un appareil dont la fonction est de stimuler les organes olfactifs des poissons. L'avantage de l'appareil réside dans le fait qu'il peut facilement contrôler la durée du stimulus ainsi que l'intervalle entre deux stimuli, séparément ou en succession. Avec légère modification, le dispositif peut s'adapter aux organes du goût.

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WITH the exception of the work of Satou (1971), who investigated adaptation mechanism in the olfactory bulb, exclusively manual techniques have been employed for controlling the delivery of chemical stimulants to olfactory organs of fishes.

In testing the effects of water-borne pollutants on the olfactory systems of fishes, it became necessary for us to develop a technique to record electrical response from the olfactory bulbs for extended periods of time.

The following describes a technique that has been successfully employed on the olfactory systems of rainbow (*Salmo gairdneri*) and brook (*Salvelinus fontinalis*) trout. It could be used on other fish species and, also, with slight modification, on the gustatory systems.

Apparatus — The device consists of a delivery apparatus and an electronic time switch. The delivery apparatus (Fig. 1) is composed of a pair of three-way valves (Chromatronix Type CAV-3060; see also Wang and Bernard 1970), which have flow passages connected as a tee in one position (from right to left in the figure) and the top and the left parts connected in a second position. Switching of these valves can be quickly done by automatic operation of pneumatic actuators (Chromatronix Type PA-875) and spring return actuators (Chromatronix Type SR-1) attached to the valves. To establish electrical control of the pneumatic actuators, the solenoid valves (Chromatronix Model SOL-3-24 V d-c; SV-1 and 2 in Fig. 1) are used. They are three-way valves which pressurize the actuators when electrical power is on and vent the actuators when power is off. Exact timing of the actuation of the solenoid valves is accomplished by an electronic time switch (TS in Fig. 1).

The circuit diagram of the electronic timer is shown in Fig. 2. The automatic sequence is started by turning

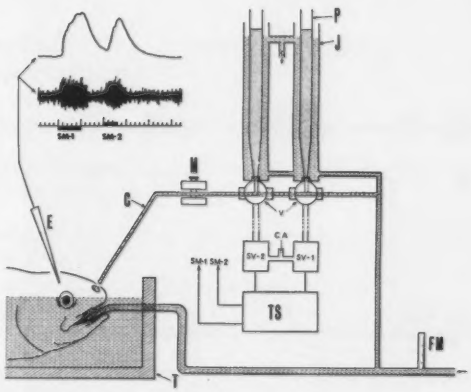

FIG. 1. Experimental setup for recording olfactory bulbar responses. Stimulant solutions are drawn into glass pipettes (P) and placed in holding tubes (J). By switching the three-way valves (V), stimulants are led onto both nares through a pair of glass capillaries (C) adjusted by a manipulator (M). CA, compressed air; E, recording electrodes; FM, flow meter; SM, signal markers; SV, solenoid valves; T, trough; TS, time switch.

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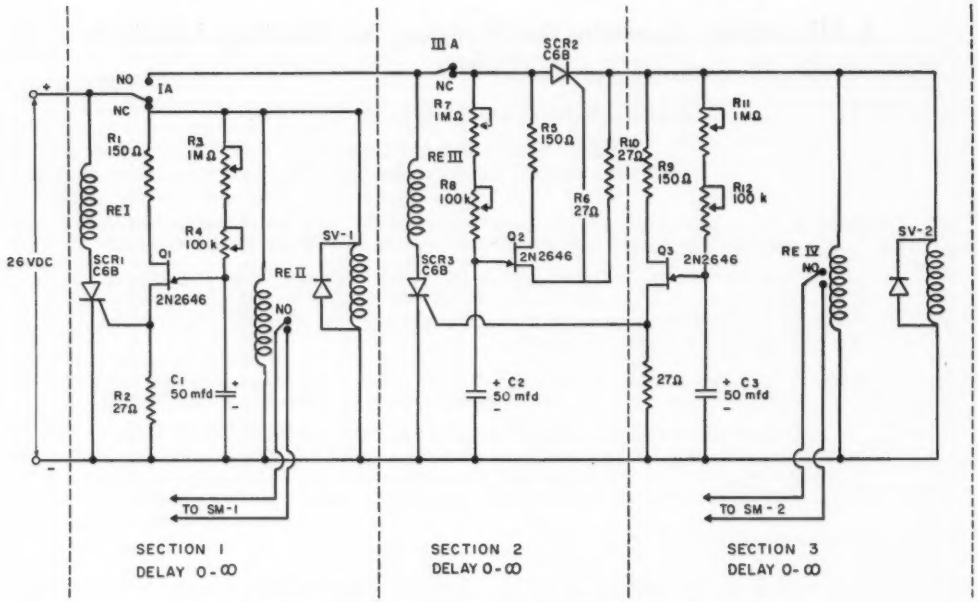

FIG. 2. Circuit diagram of the time switch. See text for the details.

on the 26-v d-c supply. When the power is turned on, solenoid SV-1 and relay RE II are energized, and the capacitor C_1 begins charging.

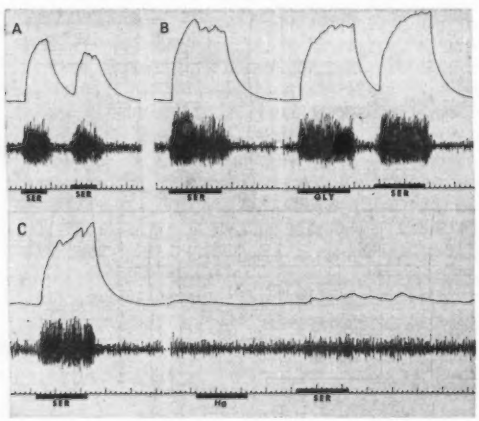

FIG. 3. Examples of typical application of the apparatus to the olfactory bulbar responses. A, 10^{-5}-M L-serine solution was given repetitively with an interval of 5 sec (selfadaptation). B, responses to 10^{-4}-M L-serine before and after 10^{-4}-M glycine was given (crossadaptation). C, Effect of 10^{-4}-M $HgCl_2$ on the bulbar response. Period of stimulation and time intervals of 1 sec are shown below each record. Calibration, 50 μv.

When unijuction transistor Q_1 fires it turns on SCR_1, which in turn applies power to RE I and moves contact IA to the NO position. SV-1 and RE II are de-energized and power is now applied to section 2. C_2 starts charging through the NC contact of III A. When C_2 is charged it fires Q_2, which turns on SCR_2 and applies power to section 3. SV-2 and RE IV are energized and C_3 starts charging. When charged it fires Q_3, which fires SCR_3, and power is now applied to RE III breaking contact III A, thus completing the cycle. RE II and RE IV are separately connected to the terminals of the signal marker pens (SM-1 and SM-2) of the recorder for event marking.

The time constants (0–∞) in each section are determined by the two potentiometers and the 50-μf capacitor in the emitter leg of the unijunction transistors.

Experiment — Operative procedures of the fish and recording techniques of the electrical activity are described elsewhere (see Hara 1972). Constant volumes of stimulant solutions were drawn into disposable glass pipettes (P), using a preset automatic syringe, and placed in the holding tubes (J); between them water circulated continually to minimize temperature difference. Thus, possible contamination of two solutions in both holding tubes could also be avoided. Stimulants and infusing water are led onto both nares through a pair of glass capillaries (C) adjusted by a manipulator (M).

Upon turning on the time switch as described above, flow of the infusing water to the nares is immediately interrupted by a flow of a stimulant solution. The flow rate depends on the height of solution in the pipette. When repetitive stimuli are to be given at the same rate,

the pipettes can be easily connected to large reservoirs of stimulant solutions using vinyl tubings. The duration of the stimuli and the interval between them are predetermined. The infusing water is completely replaced by a stimulant solution in 0.3 sec, at the least, and it is washed out in 2 sec, as determined by conductivity measurement. The main factor limiting this time delay is the length of the glass capillaries leading the stimulant solution to the nares.

Some examples of typical application of the apparatus are shown in Fig. 3. In "A" two individual stimuli (10^{-5}-M L-serine for 5 sec) were given with an interval of 5 sec. It is thus possible to examine the time course of recovery of the system for a test response following a constant or varying adaptation period. If two different stimuli are given during a certain interval, the degree of the response evoked by the testing (second) stimulus varies depending upon the difference between adapting (first) and testing stimuli. "B" is an example of such an experiment (the cross-adaptation method). This method may be of use in determining to what degree fish can discriminate the qualitative difference between two substances. Here, the responses to 10^{-4}-M L-serine before and after stimulation with 10^{-4}-M glycine are shown.

Of particular interest from a practical viewpoint is an application to the study of water pollution. For example, brief treatment of the olfactory receptor with 10^{-4}-M $HgCl_2$ solution for 10-sec blocks almost entirely the response to 10^{-5}-M L-serine (Fig. 3C).

By simple reconnecting of the paired valves in parallel, the apparatus is also used for stimulating both nares simultaneously with two different chemical stimulants or a single stimulant of different concentrations.

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HARA, T. J. 1972. Olfactory responses to amino acids in rainbow trout, *Salmo gairdneri*. Comp. Biochem. Physiol. (In press)

SATOU, M. 1971. Electrophysiological study of the olfactory system in fish. I. Bulbar responses with special reference to adaptation in the carp, *Cyprinus carpio* L. J. Fac. Sci. Univ. Tokyo Sect. IV Zool. 12: 183–218.

WANG, M. B., AND R. A. BERNARD. 1970. Adaptation of neural taste responses in cat. Brain Res. 20: 277–282.

Tunnel-Digging in Mud by Newly-Settled American Lobsters, *Homarus americanus*

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BERRILL, M., AND R. STEWART. 1973. Tunnel-digging in mud by newly-settled American lobsters, *Homarus americanus*. J. Fish. Res. Board Can. 30: 285–287.

Newly settled lobsters (stages IV through VIII) appear well adapted to living on mud. In aquaria with mud substrates, they dig U-shaped tunnels by means of a combination of plowing and fanning activities.

BERRILL, M., AND R. STEWART. 1973. Tunnel-digging in mud by newly-settled American lobsters, *Homarus americanus*. J. Fish. Res. Board Can. 30: 285–287.

Les homards post-larvaires (stades IV à VIII) semblent bien adaptés à une vie sur fond de vase. Dans des viviers à substrat de vase, ils creusent des tunnels en U en combinant labourage et battement de l'eau.

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THE behavior, habitat, and distribution of newly settled *Homarus americanus* are relatively unknown aspects of the biology of the species. However, from the studies of Cobb (1968), Hadley (1908), Herrick (1911), Scarratt (1968), and Templeman (1937) it appears that following the pelagic larval stages I–III, the stage IV lobster is both epibenthic and pelagic. It possibly is testing its substrate, settling on gravel if it finds any before molting into stage V, which appears to be the first truly settled stage.

Though settled lobsters are commonly associated with sand, gravel, and rock substrates, there are a few accounts of them living on mud. Thomas (1968) described over-wintering burrows (single-opening excavations) of mostly mature individuals in the mud and shell substrate of the sloping shores of Bideford River, P.E.I. MacKay (1920a, b) found several small individuals, 7–10 cm in total length, in tunnels (excavations with two surface openings) in the soft bottom of Richmond Bay, P.E.I.

This study was designed to determine the tunnel-digging ability of lobster stages IV through VIII in an artificial habitat with a mud substrate.

Methods — Newly hatched larvae were cultured individually in 1-liter bottles from July through August 1971. They were fed daily with *Artemia* naupliae until they molted to stage IV, and the water in each culture bottle was changed completely every second day. Water temperature varied from 15–23 C.

Subsequent stages IV through VIII were fed fragments of the mussel *Mytilus edulis* and were kept in glass-sided aquaria of 2–20 liter capacity. The water in each aquarium was changed at least once a week, and varied in temperature from 15–23 C. Each aquarium had a mud substrate 8–12 cm deep, and several stones 3–6 cm in diameter were placed on the mud surface. Of the lobsters observed in these aquaria, 30 were stage IV, 18 stage V, 10 stage VI, 6 stage VII, and 3 stage VIII.

Results — The 30 stage IV lobsters averaged 11.9 mm long, and spent an average of 13 days in stage IV before molting to stage V. Of these 30 individuals, 27 dug their first tunnels during an 8-day period commencing 2 days after the molt to stage IV. Of the remaining three individuals, two burrowed during their first day in stage IV and one did not burrow until it molted to stage V. The tunnels varied in depth and in the distance apart of the two surface openings, but only those excavated against the glass side of an aquarium could be measured with any accuracy. All such tunnels were U-shaped, without side compartments or extensions, with a mound of excavated material deposited just beyond either opening. No tunnel was dug deeper than 3 cm or was longer than 5 cm between openings.

All individuals of stages V through VIII (13.6–23.0 mm long) began tunnel-digging within 4 hr of being placed on a mud substrate, and their tunnels were proportionately larger than those of stage IV. On several occasions a dead-end side chamber was dug off the bottom of the tunnel, and in two instances a third surface opening was made. The largest tunnel made by a stage VIII lobster (21.5 mm long) was 9 cm deep and 12 cm between surface openings.

If the mud had no surface stones, a young lobster usually dug in one corner of its aquarium, but when stones were present, the tunnel was usually under the edge of one of them. By placing a stone against the side of an aquarium, the lobster could be induced to dig against the glass. Figure 1 illustrates stages IV and VIII lobsters involved in maintaining such tunnels which they recently finished digging.

Each lobster stage began digging a tunnel by loosening the mud with its pincers and pulling the loosened mud free. Mud was then removed either by plowing or fanning. When plowing, the first two pairs of walking legs carried the load of debris, the third pair pushed more debris into the load, and the last pair was used for walking. The chelipeds were usually extended around the load. When fanning, the lobster ejected the loosened mud from its tunnel by standing over the debris with abdomen raised and telson extended. Then, by vigorous beating of the swimmerets, a current was created that carried the loose mud rapidly backwards usually as far as the tunnel opening.

Plowing and fanning were the only digging methods used by almost all individuals. However, on at least two occasions stage VII lobsters backed down the tunnel, scooped up a load of mud by curling the telson around it, and then carried the load held between the telson and last pair of walking legs to the top of the discard mound.

Lobsters remained in their tunnels unless driven out by others or the tunnel collapsed. When food was placed in the aquarium, a lobster would emerge from its tunnel, find and grasp a piece, and then rapidly retreat to its tunnel. Though individuals defended their tunnels against other lobsters, there was no sign of true territoriality.

Discussion — Stages IV through VIII of *H. americanus* appear well adapted for living on a mud substrate, digging tunnels in a similar way to those dug by *Nephrops norvegicus*, a species which typically is found on a mud substrate (Rice and Chapman 1971). The mud must be firm enough so that tunnels do not collapse too easily, and it probably must have stones or shells scattered on its surface to act as objects for the lobsters to begin their tunnels under. Cobb's observations (1968) are interesting in this context, for he found that stage IV lobsters were significantly later in molting to stage V when cultur-

FIG. 1. A, Stage IV lobster, at a surface opening. It has just pushed the small dark stone up from the bottom of the tunnel; B, Stage VIII lobster, at bottom of tunnel, about to plow loose mud to the surface; C, Same individual as in Fig. 1B, fanning loose mud from under its raised telson.

Berrill and Stewart — J. Fish. Res. Board Can.

ed over a mud substrate (lacking surface stones) than when cultured over gravel. Though this implies that stage IV lobsters prefer gravel to settle on and hide in, there may be a great difference between mud with surface objects to induce digging, and mud without such objects.

Though it is tempting to speculate that newly settled lobsters do in fact prefer a mud substrate to sand, gravel or rocky ones, there are few supporting field observations beyond the brief ones of MacKay (1920a, b). Moreover, newly settled lobsters are also capable of burrowing in sand under stones, and of hiding themselves in gravel spaces. Tunnel-digging is, however, a relatively complex activity, and should play a potentially essential role in the successful survival of newly settled lobsters to account for its evolution.

Acknowledgments — This research was supported by NRC Grant #A5447.

COBB, S. J. 1968. Delay of moult by the larvae of *Homarus americanus*. J. Fish. Res. Board Can. 25: 2251–2253.

HADLEY, P. B. 1908. The behavior of the larval and adolescent stages of the American lobster (*Homarus americanus*). J. Comp. Neurol. Psychol. 18: 199–301.

HERRICK, F. H. 1911. Natural history of the American lobster. Bull. U.S. Bur. Fish. 29: 149–408.

MACKAY, D. A. 1920a. Report upon yearling and two year old lobsters in Richmond Bay, P.E.I. for 1920. Can. Dep. Nav. Serv. Atl. Biol. Sta. Annu. Rep. 49: 1–13.

1920b. Post-larval lobsters. Science 64: 530.

RICE, A. L., AND C. J. CHAPMAN 1971. Observations on the burrows and burrowing behavior of two mud-dwelling decapod crustaceans, *Nephrops norvegicus* and *Goneplax rhomboides*. Mar. Biol. (N.Y.) 10: 330–342.

SCARRATT, D. 1968. Distribution of lobster larvae (*Homarus americanus*) off Pictou, Nova Scotia. J. Fish. Res. Board Can. 25: 427–430.

TEMPLEMAN, W. 1937. Habits and distribution of larval lobsters (*Homarus americanus*). J. Biol. Board Can. 3: 343–347.

THOMAS, M. L. H. 1968. Overwintering of American lobsters, *Homarus americanus*, in burrows in Bideford River, P.E.I. J. Fish. Res. Board Can. 25: 2725–2727.

An Improved Ekman-Type Grab

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BURTON, W., AND J. F. FLANNAGAN. 1973. An improved Ekman-type grab. J. Fish. Res. Board Can. 30: 287–290.

An improved Ekman-type grab with top lids, which are locked open during descent and weighted to keep them closed during retrieval, is described. In comparative trials with a Wildco Tall 6-inch Birge-Ekman, the new grab collected significantly more chironomids and oligochaetes, and about the same number of the heavier and larger animals such as gastropods, sphaeriids, trichopterans, etc. The new design also improves the versatility and mechanical reliability of the grab.

BURTON, W., AND J. F. FLANNAGAN. 1973. An improved Ekman-type grab. J. Fish. Res. Board Can. 30: 287–290.

Nous décrivons un modèle amélioré de benne d'Ekman avec couvercles supérieurs maintenus ouverts durant la descente par un enclenchement, et fermés à l'aide de poids durant la remontée. Au cours d'essais comparatifs avec une benne Wildco Tall Birge-Ekman de 6 pouces, le nouveau modèle a recueilli nettement plus de chironomides et d'oligochètes, et à peu près le même nombre d'organismes plus lourds et plus gros tels gastéropodes, sphaeriidae, trichoptères, etc. De plus, le nouveau modèle est plus versatile et mécaniquement plus sûr que l'ancien.

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THE messenger-released version (Birge 1922) of Ekman's (1911) grab is perhaps the most widely used single sampler in studies of freshwater macrobenthos. However, the models commercially available in North America suffer from several defects. Brinkhurst (1967) has outlined most of these and Flannagan (1970) added to the list. The defects may be summarized as follows: the sampler does not have a mechanism which will retain the lids in a vertical position during descent and thus the water pressure required to hold the lids open will contribute to the hydraulic shock wave on impact with the substrate; screening in the upper sample chamber will cause further pressure build up; there is no weight or lock-down system incorporated on the lids, thus loss of animals during retrieval is likely; the tripping mechanism for the jaws is subject to failure or premature release; construction methods and materials are not sufficiently strong to withstand normal usage.

The sampler described below was designed to overcome these problems. It is constructed of stainless steel, has a locking system for holding the lids open during descent, and these lids are weighted to minimize "lid flap" during retrieval. It has a new, more reliable, jaw-release system; in addition, it has the following advantages — the jaw pivots are manufactured in one piece, it has no limitations to height of the sample chamber, and a guard is fitted over the release mechanisms.

Construction Details — The grab is illustrated in Fig. 1. All parts, with the exception of the springs,[1] were made from stainless steel.

The sample chamber — (Fig. 2A) was fabricated from 16 standard wire gauge (s.w.g.) sheeting and welded along the joint. The height of the chamber is 9 inches with inside dimensions of 6 × 6 inches and a $\frac{1}{16}$-inch wall thickness.

The jaws — (Fig. 2B) were constructed from 16 s.w.g. sheeting and welded along the joints. The leading edges were sharpened to ensure a better cutting action. Four lugs (Fig. 2C), for the attachment of the main spring legs, were machined and flush riveted in position.

Two jaw stop bars (Fig. 2D) were machined from $\frac{1}{2}$ × $\frac{1}{4}$-inch flat bar and held in position with 10–32 diameter screws. The stop bars prevent the sharpened edges of the jaws from being blunted, by simultaneously striking the sample chamber when the jaws close, and also prevent the jaws from overriding their central position.

To facilitate opening the jaws, two handles (Fig. 2E) were made from $\frac{3}{16}$-inch diameter rod and held in place by 10–32 diameter nuts. Two bearings (Fig. 2F), on which the jaws and main springs hinge, were connected to the sample chamber by $\frac{1}{4}$-inch diameter bolts and retained with lock nuts.

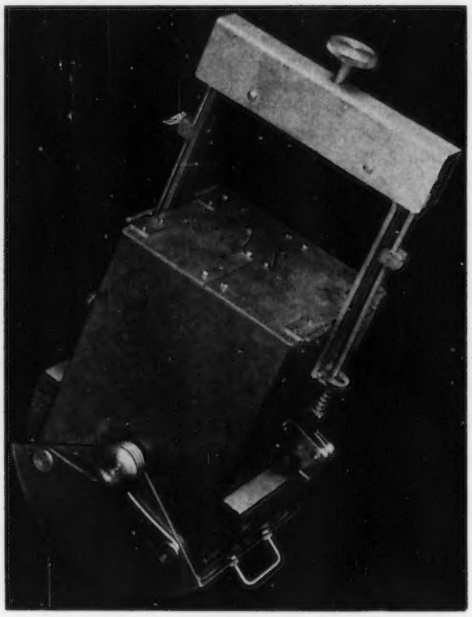

FIG. 1. The improved Ekman-type grab.

The bridge, release mechanism, and guard — The bridge (Fig. 2G) was formed from $\frac{3}{16}$ × 1-inch diameter flat bar and connected to the sample chamber by 10–32 diameter screws. The lower ends of the bridge were bent out at right angles, and two $\frac{1}{4}$-inch diameter elongated holes were drilled to serve as guides for the side rods (Fig. 2H) and also to take up tension on the side rod springs (Fig. 2J).

Eight lugs were welded in position on top of the bridge. The four inner lugs (Fig. 2K) were drilled and tapped for 8–32 diameter screws, for attachment of the guard (Fig. 2M). The four outer lugs (Fig. 2L) were drilled and tapped for 10–32 diameter screwed pins on which pivot the lever arms (Fig. 2N). The side rods (Fig. 2H) comprise a $\frac{1}{4}$-inch diameter central rod, screw-cut at both ends, onto which fit two connecting links. The upper connecting links (Fig. 2Y) are coupled to the lever arms, and the lower links (Fig. 2Z) are coupled to the jaw release catch (Fig. 20) with 8–32 diameter pins.

Two small blocks (Fig. 2P) fitted to the central rods, and held in position by 6–32 diameter grub screws serve to disengage the lid springs (Fig. 2Q). Two compression springs (Fig. 2J) mounted on the central rods between the lower ends of the bridge and upper edge of the bottom connecting links create the required pressure to hold the release mechanism in the open position.

[1]Main springs were purchased from Wildlife Supply, Mich. (Type 199D for 196T Ekman).

The jaw release catches pivot on 8–32 diameter pins, screwed through the release catch housing (Fig. 2R) and retained with lock nuts.

The release catch housings were made from 16 s.w.g. sheeting and spot welded to the sample chamber.

The central tube of the release plunger (Fig. 2S) extends up through a bush welded in the center of the guard. A coil spring (Fig. 2X) over the plunger is retained with a wide, circular nut (Fig. 2T).

A rope or cable extends down through the central tube of the release plunger and passes through a $\frac{1}{4}$-inch diameter hole in the center of the bridge where it is secured with a cable clamp.

The lids — (Fig. 2U) were made form 16 s.w.g. sheeting and connected to the sample chamber with four small hinges (cut from 1.5 inch s.s. piano hinges) riveted in place. Two $\frac{1}{4} \times \frac{1}{2}$-inch weight bars (Fig. 2V) were riveted to the under side of the lids. Two small lid spring catches (Fig. 2Q) made from thin flat spring material were bolted to the top inner edge of the lids. $\frac{1}{4}$-inch diameter holes (Fig. 2W) were drilled on each side of the bridge and filed square, with a 45° angle at the bottom of the inside edge. When the lid springs pass through the hole this angle causes them to come under tension, and by virtue of the spring shape, they engage and lock on the outer edge.

Operation — The new Ekman and a tall 6-inch Birge-Ekman grab (Wildlife Supply Co. Ltd., Mich.) were used to sample the benthos of the profundal mud of Lake Winnipeg. Ten samples were taken at approximately the same spot with each grab. Samples which were overfilled were discarded, as were samples which did not fill at least two thirds of the grab; thus, all the samples were close to the size at which light or surface-dwelling animals are likely to be lost through the top flaps (Flannagan 1970). All samples were sieved through

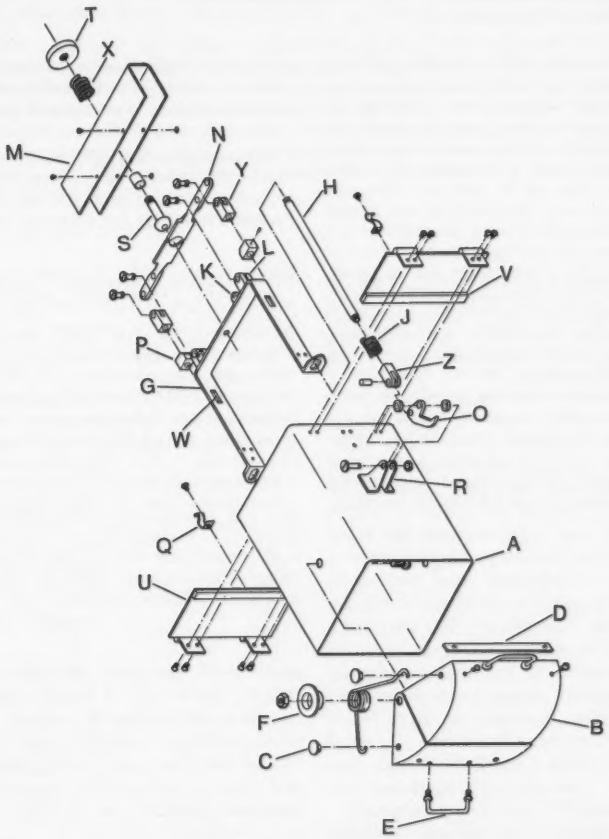

FIG. 2. Exploded isometric plan of the grab.

TABLE 1. Mean number, standard deviation, and *t*-values for the various benthic invertebrate groups collected by 10 samples with each grab.

	New Ekman Mean no./grab ± SD	Old Ekman Mean no./grab ± SD	*t*-value New Ekman vs. Old Ekman
Chironomidae	78.9±39.876	32.8±17.203	3.357[a]
Sphaeriidae	20.9±8.491	23±9.080	0.534
Oligochaeta	24.3±14.900	2.9±2.726	4.468[a]
Gastropoda	2.1±1.100	1.2±1.932	1.280
Ephemeroptera	5.2±2.898	4.5±2.068	0.624
Conchostraca	0.9±0.876	0.2±0.422	2.277[b]
Ostracoda	0.8±0.919	0.5±0.707	0.818
Trichoptera	0.2±0.422	0.4±0.699	0.774
Total Benthos	133.3±56.890	65.5±25.457	3.440[a]

[a]Difference significant at $P < 0.01$ (single tailed tests).
[b]Difference significant at $P < 0.05$.

0.2-mm screen, preserved in 10% formalin, and later sorted under lower power of a dissecting microscope.

The results of this trial (Table 1) clearly show the superiority of the new design, at least, when the grabs are more than two-thirds filled. If the grabs are not filled to this extent, any differences in numbers of benthic animals collected are likely to be minimal. The new grab collected twice as many chironomids and almost 10 times as many oligochaetes ($P < 0.01$), but did not collect more ($P < 0.05$) of the heavier animals such as sphaeriids, gastropods, etc. These results are in accordance with the suggestion by Flannagan (1970) that the loss of animals is related to the height of the sample within the sample box, is generally restricted to surface dwelling or lighter animals, and can be minimized by preventing flapping of the lids on retrieval.

This grab is a little more troublesome to cock than the Birge-Ekman in that two actions are required, the first to set the jaws and the second to set the lids. However, this disadvantage is more than offset by the more positive jaw release action, the reliability of the triggering mechanism, and the solving of the lid flapping problem. Additional weights can be attached similar to those supplied for the Wildco Birge-Ekman, but these are not generally required since the new grab is heavier.

Acknowledgments — We would like to thank Miss S. Warwick and Miss M. Roberts for assistance with the collection and sieving of the samples and Mr S. MacFarlane for sorting the preserved samples.

BIRGE, E. A. 1922. A second report on limnological apparatus. Trans. Wis. Acad. Sci. Arts. Lett. 20: 533–552.

BRINKHURST, R. O. 1967. Sampling the benthos. Great Lakes Inst. Progr. Rep. 32, Univ. Toronto, Toronto, Ont. 7 p.

EKMAN, S. 1911. Neue Apparate zur qualitativen und quantitativen Erforschung der Bodenfauna der Seen. Int. Rev. d. ges. Hydrobiol. 3: 553–561.

FLANNAGAN, J. F. 1970. Efficiencies of various grabs and corers in sampling freshwater benthos. J. Fish. Res. Board Can. 27: 1691–1700.

Intestinal Helminth Parasites of Northern Sand Lance (*Ammodytes dubius*)

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SCOTT, J. S. 1973. Intestinal helminth parasites of northern sand lance (*Ammodytes dubius*). J. Fish. Res. Board Can. 30: 291–292.

Six species of helminth parasite were found on examination of intestines of 438 specimens of northern sand lance (*Ammodytes dubius*) from various areas of the Nova Scotia Banks. They were: *Derogenes varicus*, *Brachyphallus crenatus*, *Lecithaster gibbosus*, *Bothriocephalus scorpii*, *Contracaecum aduncum*, and a cestode (tetraphyllid) larva. Intensity of infestation was low and incidence low to moderate, with little difference between areas. Parasite species composition in *A. dubius* was similar to that of *A. marinus*, its European counterpart, but unlike that of *A. americanus*, the neighboring species to *A. dubius* in the northwest Atlantic.

SCOTT, J. S. 1973. Intestinal helminth parasites of northern sand lance (*Ammodytes dubius*). J. Fish. Res. Board Can. 30: 291–292.

L'auteur a découvert six espèces d'helminthes parasites dans l'intestin de 438 spécimens d'*Ammodytes dubius* provenant de diverses régions des bancs de la Nouvelle-Ecosse. Ce sont: *Derogenes varicus*, *Brachyphallus crenatus*, *Lecithaster gibbosus*, *Bothriocephalus scorpii*, *Contracaecum aduncum*, et une larve de cestode (tétraphyllidien). Le degré d'infestation est faible et l'incidence varie de basse à modérée, sans qu'il y ait de grandes différences régionales. Le complément d'espèces parasites d'*A. dubius* est semblable à celui d'*A. marinus*, son équivalent européen, mais différent de celui d'*A. americanus*, espèce voisine d'*A. dubius* dans le nord-ouest de l'Atlantique.

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NORTHERN sand lance (*Ammodytes dubius*) is the common lance on offshore banks in the northwest Atlantic from West Greenland to New England. There are no records of its parasites. While examining gut contents of trawl caught northern sand lance from the Nova Scotia Banks in 1970, intestinal helminth parasites were collected and identified to assess their potential as biological tags or indicators for population studies.

Fish were frozen immediately after capture and later thawed in the laboratory for examination. The whole intestine from each fish was excised and opened in a petri dish under water. Contents were examined under a stereomicroscope and helminth parasites separated and preserved in 8% formalin.

Six species of helminth parasites from three different classes were found in *A. dubius*: Trematodes: *Derogenes varicus*, *Brachyphallus crenatus*, and *Lecithaster gibbosus*; Cestodes: *Bothriocephalus scorpii* and a tetraphyllid larva; and Nematode: *Contracaecum aduncum*. All occurred at very low levels of intensity and low to moderate incidence (Table 1). The only notable exceptions to the general uniformity of infestation were the relatively high incidence of *D. varicus* and *B. scorpii* on Western Bank, and the slight increase in incidence of *C. aduncum* to the north from Browns Bank to Banquereau. The tetraphyllid larva occurred as a single specimen on Browns Bank. Low intensities and high variation in incidence in different samples from the same area preclude use of the parasites as biological tags, and also indicate that *A. dubius* is not an important vector in transmission of cestodes and nematodes to other fishes.

Apart from *L. gibbosus* and *B. scorpii*, all parasites have been recorded previously from other species of *Ammodytes* although not from *A. dubius*. They are all "universal" parasites, the adults showing very low degrees of host specificity. The parasite assemblage is characteristic of fish of shallow offshore banks, feeding largely on crustaceans such as copepods, which are intermediate hosts for the trematodes, and on small fish and polychaetes which may serve as intermediate hosts for cestodes and nematodes.

Comparison of lists of parasites recorded from different species of *Ammodytes* shows obvious differences in parasite species compositions (Table

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TABLE 1. Intensity of infestation (Int) and percentage incidence (Inc) of parasites in different areas of Nova Scotia Banks. (Number of fish examined in brackets.)

Area		*Derogenes varicus*		*Brachyphallus crenatus*		*Lecithaster gibbosus*		*Bothriocephalus scorpii*		*Contracaecum aduncum*	
		Int	Inc	Int	Inc	Int	Inc	Int	Inc	Int	Inc
Banquereau	(94)	0.36	25.5	0.46	31.9	0.01	1.11	0	0	0.03	3.2
Middle	(78)	0.04	3.8	0.24	12.8	0	0	0.08	7.7	0.03	1.6
Western	(122)	0.91	48.4	0.35	27.1	0.03	1.9	0.07	3.2	0.01	1.6
Emerald	(50)	0.16	12.0	0.02	2.0	0	0	0.26	10.0	0.02	2.0
Browns	(94)	0.41	31.9	0.34	25.5	0.01	1.4	0	0	0	0

2). There is close similarity between *A. dubius* and *A. marinus*. However, the parasites' low host specificity suggests that similarity is a result of behavioral rather than genetic characteristics, although it has been suggested that *A. dubius* and *A. marinus* are conspecific (Richards 1965). The comparatively large number of trematode species in *A. americanus*, the inshore sand lance of the western Atlantic, indicates very different feeding behavior from that of neighboring *A. dubius*, and even from *A. tobianus* (= *A. lancea*?), its European counterpart.

TABLE 2. Parasites recorded from various species of *Ammodytes*.

Parasites	Species of *Ammodytes*				
	A. tobianus	*A. americanus*	*A. marinus*	*A. dubius*	*A. cicerellus*
Stephanostomum tenue		×			
Podocotyle olsoni		×			
Opecoeloides (*Cymbephallus*) *vitellosus*		×			
Lepocreadium pyriforme		×			
Brachyphallus crenatus		×	×	×	
Torticaecum fenestratum		×			
Lecithaster gibbosus				×	
Derogenes varicus			×	×	
Hemiurus communis	×				
Hemiurus appendiculatus	×				
Scolex polymorphus			×		
Bothriocephalus (*parvus*) *scorpii*	×			×	
Tetrarhynchus gracilis					×
Contracaecum aduncum			×	×	
Anisakis sp.			×		
Echinorhynchus gadi			×		

It is surprising that *B. scorpii*, the most obvious intestinal parasite, has not been previously reported. The only other adult cestode recorded from *Ammodytes* is a doubtful record of *Bothriocephalus* (*parvus*) by Creplin (1846) from *A. tobianus*. Adult *B. scorpii* found in *A. dubius* were small (up to approximately 20 cm) compared to specimens found in larger fishes and it is likely that Creplin's tentative erection of a new species, based on its small size, is in error.

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CREPLIN, F. C. H. 1846. Nachtrage zu Gurlt's verzeichniss der thiere bei welchen entozoen gefunden worden sind. Arch. Naturgesch. 12 Jahrg. Bd. 1: 129–160.

RICHARDS, S. W. 1965. Description of the postlarvae of the sand lance (*Ammodytes*) from the east coast of North America. J. Fish. Res. Board Can. 22: 1313–1317.

Mercury in the Marine Environment: Concentration in Sea Water and in a Pelagic Food Chain

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Mercury in seawater, in a pelagic food chain, and in bottom sediment was determined at a single station 430 km southeast of San Diego, California. The concentration of mercury in zooplankton slightly increased with depth of collection. The mercury content in almost all of the higher trophic levels of organisms collected at greater depths was indistinguishable from the concentration of mercury in zooplankton at these depths. Mercury concentration in the seawater column was essentially constant below 100 m and significantly higher at the surface. This vertical profile of mercury content is not ascribable to biological activity.

WILLIAMS, P. M., AND H. V. WEISS. 1973. Mercury in the marine environment: concentration in sea water and in a pelagic food chain. J. Fish. Res. Board Can. 30: 293–295.

Nous avons dosé le mercure dans l'eau de mer, dans une chaîne alimentaire pélagique et dans le sédiment benthique à une seule station située à 430 km au sud-est de San Diego, Californie. La teneur en Hg du zooplancton augmente légèrement avec la profondeur d'échantillonnage. Celle de presque tous les organismes des chaînons supérieurs de la chaîne alimentaire recueillis en profondeur est indiscernable de la teneur en Hg du zooplancton à ces profondeurs. La colonne d'eau a une teneur en Hg pratiquement uniforme au-delà de 100 m, et nettement plus élevée à la surface. Ce profil vertical de la teneur en Hg n'est pas attribuable à l'activité biologique.

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To test the premise that mercury may be increasingly concentrated along successively higher trophic levels of marine food chains and that the concentration of mercury in the seawater column is affected by biological activity, organisms, water, and sediment were collected at one open-ocean (pelagic) station (29°00.0′N, 122°31.2′W) 430 km southeast of San Diego, California, and their total mercury content was measured.

Materials and methods — Zooplankton, bathypelagic fish, and crustacea were collected with an opening–closing "Bongo" net, and benthic organisms and one sediment sample with a "Sigsby" trawl. A squid and skipjack tuna were caught at the surface with handlines. All samples were so treated that the organisms did not contact metallic surfaces, diesel fuel, or hands. These materials were frozen immediately at –20 C in acid-washed plastic bags placed in glass jars. The water samples were collected in 30-liter "Niskin" bottles, then put in 600-ml nitric-acid-washed salinity bottles and immediately frozen.

The mercury content of the organisms and sediment was determined by a modification of the neutron-activation technique of Johansen and Steinnes (1969). Approximately 1–2 g (wet weight) of material was irradiated for 1 hr at a flux of 2×10^{12} neutrons cm^{-2} sec^{-1} in the Lazy Susan of the "TRIGA" reactor at Gulf Energy and Environmental Systems, San Diego, Calif. Subsequently, after the addition of 10 mg of mercury carrier, the organic matter was oxidized according to Sjøstrand (1964). Mercury was then initially precipitated by reduction with freshly prepared stannous chloride (10-g mossy tin in 30-ml concentrated hydrochloric acid). After dissolution of the precipitate with aqua regia, the *p*H was adjusted to neutrality with ammonium hydroxide and the sulfide precipitated with hydrogen sulfide gas.

The mercury content of the sea water also was determined by neutron activation analysis (Weiss and Crozier 1972). One milligram of copper carrier (99.999% purity) was added to a weighed quantity of acidified sea water; copper was precipitated as a sulfide and mercury coprecipitated quantitatively. The precipitated

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TABLE 1. Concentration of mercury in organisms collected at various depths.

Organisms	Depth collected (*m*)	Mercury (*ppb*) Wet weight	Mercury (*ppb*) Dry weight
Zooplankton[a] 45% copepods 45% chaetognaths	30	16	123
Zooplankton[a] 60% copepods 30% chaetognaths	30	6	55
Skipjack tuna (flesh) *Euthynnus pelamis*	surface	180	720
Squid (flesh) *Ommastrophes bartamii*	surface	56	280
Zooplankton[a] 70% copepods	0–500[b]	11	79
Hatchet fish (whole) *Argyropleecus* sp.	550	22	157
Snipe eel (flesh) *Serrivomer sector*	600	26	186
Fish (10 specimens) *Cyclothone* sp.	600	34	261
Penaeid decapods 4 species	600	30	332
Mysid (whole) *Eucophia* sp.	1000	65	406
Fish (whole) *Nansenia* sp.	1000	22	122
Fish (flesh) *Bathylogus* sp.	1200	420	2800
Zooplankton[a] 45% copepods 45% chaetognaths	1200	35	388
Fish (whole) *Cyclothone* sp.	1900	55	323
Zooplankton[a] 30% chaetognaths 60% copepods	3200	36	189
Zooplankton[a] 80% copepods	0–3000[b]	47	312
Brittle star (whole) *Ophiophalis* sp.	4300[c]	163	280
Sponge (whole) unidentified	4300[c]	300	1580
Sediment sample Pelagic clay	4300[c]	18	39

[a]The remainder of these samples were composed of amphipods, ostracods, pteropods, Foraminifera, and fish eggs in differing proportions.

[b]These samples contain 10–20% specimens collected at depths shallower than the maximum depth indicated due to nonclosure of the nets in situ.

[c]Benthic organisms and bottom sediment.

sulfides were dissolved in nitric acid and irradiated as described above, after which 10 mg of mercury carrier were added. Then, the solution was radiochemically purified as before through double precipitation with stannous chloride and a final precipitation as the sulfide.

The sulfides were analyzed for neutron-induced ^{197}Hg. Measurements of the 77-kev gamma ray were made 7 days after irradiation, with a NaI(Tl) detector coupled to a 400-channel pulse height analyzer.

Mercury carrier yields were determined by reirradiation of the samples for 15 sec at the same neutron flux. Subsequently, the ^{197}Hg activity was compared to that of a mercury carrier standard. The yield of carrier was 80–90%. Procedural blanks were free of detectable quantities of ^{197}Hg. One standard deviation, previously determined for the seawater analyses, was 11.2% (Weiss and Crozier 1972). For the organisms and sediment sample, the error is of the same magnitude as found in unpublished work of Weiss and Mendola.

Results — The concentrations of mercury in the organisms (Table 1) were: (1) zooplankton taken at 30–500 m, 55–123 parts per billion (ppb); (2) zooplankton collected at 1200–3200 m, 189–388 ppb; (3) organisms less than 10 cm in length (which include hatchet fish, snipe eel, Cyclothones, decapods, mysid, and *Nansenia*), 122–406 ppb; (4) the squid and brittle star, 280 ppb each; (5) the skipjack tuna and the bathypelagic fish (*Bathylogus* sp.), 720 and 2800 ppb of mercury, respectively.

The mercury content of sea water (Table 2) near the surface and at 100 m, the extent of the upper mixed layer, was 270 and 95 parts per trillion (ppt), respectively. From 210 to 4080 m the concentration was essentially constant (average, 42 ppt).

As found at this station, surface waters often contain greater concentrations of mercury than does deeper water. For example, the average of

TABLE 2. The vertical distribution of mercury in the sea water.

Depth (*m*)	Mercury (*ppt*)
10	270
100	96
210	45
330	41
520	29
960	35
1980	42
3040	44
4080	55

data from stations in the northeastern Pacific Ocean (in the vicinity of 48°N, 127°W) also showed this relation, 40 ppt at the surface and 25 ppt at greater depth (Weiss and Mendola 1972).

The higher concentration of mercury in the surface water may be ascribable to atmospheric influx. This conclusion is supported by the following data: mercury was incorporated in the snows of Greenland and the Antarctic at concentrations of 65 and 75 ppt, respectively, in historical times prior to the introduction of measurable quantities of mercury into the environment by man (Weiss et al. 1971). Snows deposited in Greenland in more recent times contain about twice this concentration of mercury. The average content of mercury in rainwater of the 1930s was 200 ppt (McCarthy et al. 1970). Thus, if these concentrations also are in the precipitation that falls on the sea surface, they explain the significantly greater concentrations measured in the mixed layer than in those farther below.

Biological activity does not account for the vertical profile in the sea water. The net carbon fixed by phytoplankton is 3.6×10^{16} g of carbon per year (Ryther 1963) and, if phytoplankton contain 400 ppb of mercury per gram of carbon (G. Knauer personal communication), then the mercury fixed is 1.4×10^{10} g/year. If the average mercury content in precipitation is taken as 100 ppt and the annual precipitation over the oceans is 3×10^{17} liter, then about twice as much mercury is introduced into the oceans as is fixed by the phytoplankton. Furthermore, the quantity of mercury introduced into the oceans annually is only about 1% of the mercury in the mixed layer (100 m depth), assuming an average concentration of 100 ppt as in surface waters (Robertson 1970).

Discussion — The results of this study indicate that mercury is concentrated along successively higher levels in the marine food chain by relatively small factors, if at all. Zooplankton collected at 30–500 m possess a lesser mercury content than do zooplankton taken at greater depth. However, in most cases mercury in the higher trophic levels of organisms collected at greater depth was not significantly different from the concentration of mercury in zooplankton at these depths.

Relatively high mercury levels in the skipjack tuna, the bathypelagic fish (*Bathylogus*), and the benthic sponge, all of which are long-lived organisms, is perhaps the consequence of the accumulation of mercury within the organism as a function of time.

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JOHANSEN, O., AND E. STEINNES. 1969. A simple neutron activation method for mercury in biological material. Int. J. Appl. Radiat. Isotop. 20: 751–755.

MCCARTHY, J. H., JR., J. L. MEUSCHKE, W. H. FICKLIN, AND R. E. LEARNED. 1970. Mercury in the environment. U.S. Geol. Surv. Prof. Pap. 213: 37–39.

ROBERTSON, D. E. 1970. Mercury content of Atlantic Ocean waters. Batelle Northwest Lab. Rep. SA-2968: 1–9.

RYTHER, J. H. 1963. Geographic variations in productivity, p. 129–164. *In* M. N. Hill [ed.], The sea. Vol. 2. Interscience, Publishers, Inc., New York, N.Y.

SJØSTRAND, B. 1964. Simultaneous determination of mercury and arsenic in biological and organic material activation analysis. Anal. Chem. 36: 814–819.

WEISS, H. V., AND T. E. CROZIER. 1972. The determination of mercury in seawater by radioactivation. Anal. Chim. Acta. 58: 231–233.

WEISS, H. V., M. KOIDE, AND E. D. GOLDBERG. 1971. Mercury in a Greenland ice sheet: evidence of recent input by man. Science (Washington) 174: 692–694.

Nutritional Factors in the Biochemical Pathology of Corynebacterial Kidney Disease in the Coho Salmon (*Oncorhynchus kisutch*)

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WEDEMEYER, G. A., AND A. J. ROSS. 1973. Nutritional factors in the biochemical pathology of corynebacterial kidney disease in the coho salmon (*Oncorhynchus kisutch*). J. Fish. Res. Board Can. 30: 296–298.

The influence of diet ingredient on the morbidity and biochemical pathogenesis of corynebacterial kidney disease was investigated using juvenile coho salmon (*Oncorhynchus kisutch*) fed the Abernathy dry ration made up with either corn gluten or cottonseed meal (isoprotein, isocaloric substitution). Evaluation of incidence of infection, pituitary activation and aspects of carbohydrate metabolism, acid-base balance, renal function, and hematopoietic activity showed that the actual disease incidence was about the same for both diets but the nonspecific stress of infection was more severe in fish fed the corn gluten.

Discriminant function calculations combining four physiological parameters gave a probability of 0.86 for successfully diagnosing infected fish on the basis of these blood chemistry tests.

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Nous avons étudié l'influence du régime alimentaire sur la morbidité et la pathogénèse biochimique de la maladie corynébactérienne du rein chez de jeunes saumons coho (*Oncorhynchus kisutch*) nourris d'une ration sèche d'Abernathy, constituée soit de gluténine de maïs, soit de farine de graine de coton (isoprotéine, substitution isocalorique). L'évaluation du pourcentage d'infection, de l'activation de l'hypophyse et des aspects du métabolisme des hydrates de carbone, de l'équilibre acide–base, de la fonction rénale et de l'activité hématopoïétique démontrent que l'incidence de la maladie est à peu près semblable avec les deux régimes, mais que le stress non spécifique d'infection est plus sévère chez les poissons nourris à la gluténine de maïs.

L'analyse des fonctions discriminantes combinant quatre paramètres physiologiques donne une probabilité de 0.86 d'un diagnostic exact d'infection basé sur ces essais de la chimie du sang.

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PRESUMPTIVE evidence exists that the incidence of chronic corynebacterial kidney disease in salmonid fishes is related to dietary and environmental factors. Feeding dry diets containing corn gluten appears to increase mortality from kidney disease in Pacific salmon (*Oncorhynchus spp.*), while substituting cottonseed meal for corn gluten confers protection (Wood 1968). Warren (1963) has shown that the severity of the disease is also inversely correlated with the total water hardness.

In a previous physiological study of kidney disease, Hunn (1964) found significant changes in parameters associated with hematopoietic activity and liver and kidney function in infected brook trout (*Salvelinus fontinalis*) from a hard water area. Thus, it seemed likely that a comparison of the pathological physiology of kidney disease in a salmonid held in soft water and fed a dry diet containing either corn gluten or cottonseed meal might clarify some of the dietary and environmental factors involved and allow an evaluation of the diagnostic significance of any blood chemistry changes occurring.

Materials and methods — Four groups of about 250 juvenile coho salmon (*O. kisutch*) were held at 10 C in water of 20 ppm ($CaCO_3$) total hardness. Two groups were fed the normal Abernathy dry diet containing cottonseed meal (Fowler and Burrows 1971) and two groups were fed the Abernathy diet made up on an isocaloric, isoprotein basis with corn gluten. Both diets were fed at 2% of the body weight for 1 month prior to the experiment. To initiate the disease, infected viscera was fed daily for a 1-week period in addition to the regular ration. Control groups received only the corn gluten or cottonseed meal diets. As the infection developed, feeding activity was reduced somewhat and the feeding rate was cut back accordingly.

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Pituitary activation, carbohydrate metabolism, acid–base balance, hematopoietic activity, and renal function of infected and noninfected fish were evaluated by determinations of interrenal vitamin C, liver glycogen, blood glucose, *p*H, hemoglobin (Hb), hematocrit (Hc), and blood urea nitrogen (BUN). Sampling techniques, anesthesia, and biochemical analyses were by previously described methods (Wedemeyer and Chatterton 1971). Due to the long incubation period and chronic nature of the infection, 10-fish subsamples were taken from each group, at weekly intervals, for about 6 months and all blood and tissues were identified as being from an infected (+) or noninfected (–) fish by use of gram-stained kidney smears. Some ascites and exophthalmos developed, but few of the fish sampled were frankly moribund. Multiway analysis of variance and stepwise discriminant function calculations were done on an IBM 360 digital computer through the courtesy of Dr D. Worlund, National Marine Fisheries Service, Seattle, Washington.

Results and discussion — Means and standard errors for the blood and tissue biochemical analyses together with sample size, diet composition, and disease incidence are summarized in Tables 1 and 2. As shown in Table 1, the total incidence of infection was almost the same in both diet groups. In Table 2, the significance tests are shown under "disease," "diet," and "interaction" where "interaction" is taken to mean that the difference in the particular blood or tissue chemistry between diseased or normal noninfected fish was not the same for the two diets; that is, the biochemical pathogenesis was diet dependent.

As expected (Table 2), all of the physiological characteristics tested were significantly ($P = 0.05$) affected by the disease process. Moderately elevated BUN levels in the infected fish indicated that some loss of renal function had occurred. Hematopoietic activity was also depressed somewhat, as shown by reduced Hc and Hb levels in the infected fish. Evidently, the kidney histological changes reported by Wood and Yasutake (1956) have physiological meaning even though visceral granuloma may have confused the clinical signs in their study (Bullock et al. 1971).

TABLE 1. Effect of diet ingredient on susceptibility to corynebacterial kidney disease. Juvenile coho salmon, Abernathy dry diet; isoprotein, isocaloric substitution, 10 C. There were no significant differences ($P = 0.05$).

Diet ingredient	Total no. fish	No. infected	No. noninfected	Total disease incidence (%)
Corn gluten	532	111	421	20.8
Cottonseed meal	543	112	431	20.6

TABLE 2. Effect of diet ingredient on the biochemical pathogenesis of corynebacterial kidney disease. Juvenile coho salmon, 10 C, Abernathy dry diet with corn gluten (C.G.) or cottonseed meal (C.M.); isoprotein, isocaloric substitution. Data given as mean ± standard error for the indicated sample size. * indicates statistical significance ($P = 0.05$).

Clinical chemistry	Diet ingredient	Sample size		Clinical chemistry		Significance tests		
		Infected	Noninfected	Infected	Noninfected	Interaction	Diet	Disease
Plasma chloride	C.G.	13	46	126.0±2.3	129.5±0.8	*	*	*
(*meg/liter*)	C.M.	11	55	120.0±4.7	127.0±0.6	*	*	*
Hematocrit	C.G.	13	62	23.1±2.0	36.2±0.9	*	*	*
(%)	C.M.	18	51	18.8±1.3	35.1±1.2	*	*	*
Blood *p*H	C.G.	12	43	7.37±0.37	7.32±0.15			*
	C.M.	10	58	7.37±0.35	7.26±0.13			*
Interrenal vitamin C	C.G.	8	27	139.5±21.7	236.7±19.5	*		*
(*μg/100 g*)	C.M.	8	35	178.3±23.1	257.6±18.0	*		*
Hemoglobin	C.G.	14	41	4.7±0.3	6.6±0.2			*
(*g/100 ml*)	C.M.	9	45	5.1±0.4	6.7±1.3			*
Liver glycogen	C.G.	16	63	2.4±0.9	39.9±0.7			*
(*g/100 g*)	C.M.	18	52	3.9±1.6	36.5±4.4			*
Blood glucose	C.G.	17	69	57.8±10.0	64.3±2.6	*		*
(*mg/100 ml*)	C.M.	18	66	50.2±10.1	67.3±2.1	*		*
Blood urea nitrogen	C.G.	18	70	3.7±0.9	1.8±0.2		*	*
(*mg/100 ml*)	C.M.	20	69	3.9±0.3	2.7±0.2		*	*

The acid-base balance, as indicated by blood *p*H and plasma Cl^- levels, was shifted towards algalosis though not to any substantial degree, presumably a reflection of the role of the teleost kill in ionoregulation.

The effect of kidney disease on carbohydrate metabolism, as shown by the depleted blood glucose and liver glycogen, can probably be partly explained by the reduced food intake of these chronically infected fish. There was some hypoglycemia evident in the diseased coho, but liver glycogen levels were an order of magnitude less than in the uninfected (control) fish. Apparently, the attempt to maintain blood glucose levels was at the expense of liver glycogen, which was not being replaced.

Interrenal vitamin C was substantially depleted in the diseased fish on both diets; presumably a reflection of the nonspecific stress of infection. Disrupted vitamin C metabolism has been described in several bacterial infections of higher vertebrates and is not simply due to inanition (Kendler and Harry 1967).

The effects of diet alone on the blood chemistry were significant statistically ($P = 0.05$) in only three cases: plasma Cl^-, Hc, and BUN. Corn gluten slightly elevated plasma Cl^- levels and Hc but depressed BUN (protein metabolism) in both infected and normal salmon.

Further analysis of the data indicated that diet had some influence on the biochemical pathogenesis of the infection. The interaction between disease and diet was significant for Hc, plasma Cl^-, glucose, and interrenal vitamin C. The biological meaning of these disease-diet interactions is not clear in the case of the first three parameters especially since diet alone also influenced Hc and plasma Cl^-, but the increased ascorbate depletion in the corn gluten group indicates that the stress of infection was more severe in these fish. In addition, there may be an increased vitamin C requirement during stress in fishes as well as in the higher vertebrates where it is known, for example, that ascorbate deficiency is synergistic with infections (Scrimshaw 1966; Baker 1967; Wedemeyer 1969). However, evaluating possible beneficial effects of a high vitamin C diet in chronic kidney disease was outside the scope of this study.

Thus, the previously reported increased mortality from kidney disease in salmonids fed corn gluten substituted diets can be at least partly understood on the basis of the present results. The diet apparently does not influence the actual incidence of infection, but the nonspecific stress of this chronic disease is evidently more severe in fish fed the corn gluten and thus increased mortalities occur. In support of this, recent work has shown that the Abernathy diet made up with corn gluten gives poorer growth and is a less satisfactory ration than the equivalent cottonseed meal formulation (Fowler and Burrows 1971).

Along similar lines it is tempting to speculate that one of the factors responsible for the chronic kidney disease problems in hatcheries with soft water (Warren 1963) may be the greater energy required for osmoregulation in waters of low total hardness. In an acute infection, the influence of dietary and environmental factors would probably be much reduced.

The feasibility of using the clinical chemistry profile obtained in this study as a diagnostic tool was also investigated. Under the assumption that all the variables were independent, a discriminant function of the form $Y = \lambda_1X_1 + \lambda_2X_2 + \lambda_3X_3 + \lambda_4X_4$, combining interrenal ascorbate, Hb, liver glycogen, and BUN, was calculated for both infected and noninfected fish. For this particular combination of physiological parameters the probability of successfully separating populations of healthy fish from fish with kidney disease was calculated to be 0.86, a value high enough to suggest that additional development of this technique would be worthwhile.

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BAKER, E. M. 1967. Vitamin C requirements in stress. Amer. J. Clin. Nutr. 20: 583–590.

BULLOCK, G. L., D. A. CONROY, AND S. F. SNIESZKO. 1971. Diseases of fishes. T.F.H. Publications, Newark N.J. 151 p.

FOWLER, L. G., AND R. E. BURROWS. 1971. The Abernathy salmon diet. Progr. Fish-Cult. 33: 67–75.

HUNN, J. B. 1964. Some patho-physiologic effects of kidney disease in brook trout. Proc. Soc. Exp. Biol. Med. 117: 383–385.

KENDLER, J., AND E. G. HARRY. 1967. Systemic (*Escherichia coli*) infection as a physiological stress in chickens. Res. Vet. Sci. 8: 212–218.

SCRIMSHAW, N. S. 1966. Synergistic and antagonistic interactions of nutrition and infection. Fed. Proc. 25: 1679–1681.

WARREN, J. W. 1963. Kidney disease of salmonid fishes and the analysis of hatchery waters. Progr. Fish. Cult. 25: 121–131.

WEDEMEYER, G. 1969. Stress-induced ascorbic acid depletion and cortisol production in two salmonid fishes. Comp. Biochem. Physiol. 29: 1247–1251.

WEDEMEYER, G., AND K. CHATTERTON. 1971. Some blood chemistry values for the juvenile coho salmon (*Oncorhynchus kisutch*). J. Fish. Res. Board Can. 28: 606–608.

WOOD, E. M., AND W. T. YASUTAKE. 1956. Histopathology of kidney disease in fish. Amer. J. Pathol. 32: 845–857.

WOOD, J. W. 1968. Diseases of Pacific salmon: their prevention and treatment. C 5.1. Wash. Dep. Fish., Olympia, Wash. 85 p.

Age, Growth, and Maturity of Round Whitefish (*Prosopium cylindraceum*) From the Leaf River, Ungava, Quebec

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JESSOP, B. M., AND G. POWER. 1973. Age, growth, and maturity of round whitefish (*Prosopium cylindraceum*) from the Leaf River, Ungava, Quebec. J. Fish. Res. Board Can. 30: 299–304.

Data on the age, growth, and maturity of round whitefish (*Prosopium cylindraceum*) from the Leaf River, Ungava, generally confirmed or extended observations previously reported for two other Ungava rivers. On the basis of back-calculated lengths, Leaf and Koksoak River round whitefish had similar growth rates except that the Leaf River specimens showed an atypical decrease in the second year. Scale increments in the year of the study suggested that the growing season extended from mid-June to mid-September. Both sexes matured at ages 4–6, but proportionately more females than males were mature at ages 4 and 5. Females matured at lengths between 190–199 mm and 240–249 mm while males matured at lengths 10–20 mm greater, as in the Koksoak River. Apparently, not all mature females spawned annually.

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Les données recueillies sur l'âge, la croissance, et la maturité du ménomini rond (*Prosopium cylindraceum*) de la rivière aux Feuilles, Ungava, confirment d'une façon générale ou complètent des observations faites dans deux autres rivières de l'Ungava et déjà publiées. Sur la base de rétrocalculs des longueurs, les ménominis de la rivière aux Feuilles ont le même rythme de croissance que ceux de la rivière Koksoak, sauf que les individus de la rivière aux Feuilles accusent une diminution anormale du taux de croissance dans leur deuxième année. La croissance de l'écaille durant l'année où s'est poursuivie cette étude nous porte à croire que la saison de croissance s'étend de la mi-juin à la mi-septembre. Les deux sexes atteignent la maturité sexuelle à l'âge de 4 à 6 ans, mais proportionnellement plus de femelles sont mûres à l'âge de 4 et 5 ans. Les femelles atteignent la maturité à des longueurs allant de 190–199 à 240–249 mm, alors que les mâles deviennent mûrs à des longueur de 10–20 mm plus grandes, tout comme dans la rivière Koksoak. Toutes les femelles mûres, semble-t-il, ne fraient pas annuellement.

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THE round whitefish (*Prosopium cylindraceum*) has been less intensively studied than most other North American coregonids. A report on age and growth of this species in the Koksoak and George Rivers is the only one currently available from Ungava (Mackay and Power 1968). This note on round whitefish from the Leaf River describes a more extensive study of a totally unexploited population, confirms several observations by Mackay and Power (1968), and provides additional insights into the life history of this species in Ungava. Comparisons are also made with studies conducted in the Great Lakes.

Jessop et al. (1971) describe the river system and provide details of the fish species present.

Materials and general methods — The study is based on a total of 807 round whitefish caught in the Leaf River between June 28 and September 8, 1968. Gillnets of monofilament nylon ranging from 0.5- to 2.5-inch stretched mesh in 0.5-inch intervals were used to capture 579 of the larger specimens while fry and fingerlings were taken by beach seine (161 specimens) and rotenone (67 specimens). From five to eight nets of several mesh sizes were set from shore at various locations and allowed to fish 24 hr/day throughout the study period. Most fish of the intermediate size (approximately 15–30 cm) were caught in the 1–1½-inch mesh nets, while the ½-inch mesh nets took 9–13-cm fish and the 2–2½-inch nets caught the largest fish. The seine and rotenone methods were not as frequently employed as the gillnets, nor used in the same locations.

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Fork lengths were measured to the nearest millimeter. Weights of the larger fish were determined fresh to the nearest gram, while preserved fry were weighed to the nearest 0.01 g, after damp drying. No length or weight correction factors were used for specimens measured after preservation (originally in 10% formalin, later transferred to 70% isopropyl alcohol).

Sex and state of maturity were determined by gross or microscopic examination of the gonads. Maturity was judged on Kiselevic's six category system (Nordeng 1961).

Scales for aging were removed from the left side of each fish, posterior to the base of the dorsal fin and 2–3 rows above the lateral line. They were mounted on plastic slides (Power 1964) and later examined, without further preparation, at magnifications to 80×. Scale ages were compared, when possible, with otolith age determinations (Jessop 1972).

Measurements along the anterior radius of enlarged scales (50×) were made to the nearest millimeter for back-calculation of fish length at a previous age. Seasonal growth was estimated for age-4 fish by comparing the present years growth with the calculated average increment for 10 year-classes for the fourth year of life.

Totals shown in the tables do not always agree because data on some specimens were incomplete.

Age and growth — The observed lengths overlapped markedly between most age groups, particularly ages 4+ to 8+ (Table 1). The length distribution (excluding age 0) approximates a normal curve with the mode at age 6+. No significant difference ($P = 0.05$) was found in mean length between males and females at any age. Females tended to live longer than males, and thus grow larger.

To avoid bias from the capture of fish during most of the growing season, the analysis of growth rate was based on calculated lengths at the time of annulus completion in each year. Calculations of the time of annulus completion in the study year using the regression method suggested by Backiel (Gerking 1966) indicated that annuli were completed by late June. Observation confirmed this conclusion.

A body–scale relation was determined in order to supply a "time of scale formation" correction factor for use in calculating lengths at the time of completion of each annulus. The equation was

$$Y = 1.0245X - 48.4973$$

where Y is the scale radius and X is the fork length of the fish ($n = 586$). The equation fitted the plotted empirical data well. The positive X intercept value suggests that the round whitefish were 48.5 mm in length when scales began forming. This is in agreement with observations on a number of fry indicating that scales in the area of scale sampling began forming at a length between 45 and 50 mm and were well developed at 55 mm.

The lengths (Table 2) at the end of each year were calculated for the sexes combined, as little difference according to sex was evident. The calculated lengths varied little between age-groups. Lee's phenomenon was not apparent.

Plotting of the relation between the distance from the last annulus to the scale margin and the date of collection for age-group 4+ showed that approximately 16% of the year's growth occurred by the first week of July, 67% by the end of July, and 90% by the first week of September. A pronounced reduction of the growth rate occurred in early August. Similar growth patterns were evident in the scales and otoliths (Jessop 1972) of four other age-groups.

The growth rate decreased progressively with increasing age except after the 10th year and in the second year, when the annual increment was the lowest of the first five years (Table 2).

At a given age, the growth increment varied moderately from year to year. For example, at age 3, the mean was 43.0 mm with a range from 38.7 mm for the 1963 year-class to 49.5 mm for the 1964 year-class. In certain years, the growth made by all ages of round whitefish tended to be consistently high or low.

The calculated growth rates of round whitefish from the Leaf River are similar to those for the Koksoak River, but the Ungava growth rates are much less than those of the Great Lakes fish between ages 0 and 4 and only become similar from age 5 onward (Fig. 1). Since the more northern populations appear to live longer, they ultimately approach the size of their southern counterparts. Round whitefish from the Leaf River and Koksoak River require 4 years to reach about 183 mm and 170 mm, respectively; those from Lake Superior attain the same length in 2 years (Bailey 1963) and those from Lake Michigan in less than 1 year (Mraz 1964).

The length–weight relation was described by the parabola $W = al^n$, where W is weight in grams, l is length in millimeters, and a and n are empirically determined constants. The logarithmic form of the equation was

$$\log W = -4.70466 + 3.2904 \log L.$$

Comparison of the calculated and observed weights reveals that the equation fits the data well except at the higher length intervals where there are few fish samples.

Length–weight data for round whitefish fry (age 0+) were fitted to an equation of the form

$$\log W = -2.23874 + 3.0336 \log L.$$

TABLE 1. Observed length frequencies by age-groups of 807 round whitefish (*Prosopium cylindraceum*) from the Leaf River, 1968.

Fork length (*mm*)	Age-group															
	0+	1+	2+	3+	4+	5+	6+	7+	8+	9+	10+	11+	12+	13+	14+	Total
20–29	4															4
30–39	85															85
40–49	107															107
50–59	8															8
60–69	1															1
70–79		1														1
80–89		1														1
90–99		6														6
100–109		1	2													3
110–119			10													10
120–129			3													3
130–139			7													7
140–149			11													11
150–159			8	1												9
160–169			–	1												1
170–179			1	7												8
180–189				21	4											25
190–199				21	19	1										41
200–209				6	36	1										43
210–219					24	8										32
220–229					12	13										25
230–239					7	19	4									30
240–249					2	21	13									36
250–259					1	13	28	5								47
260–269					1	5	27	14	1							48
270–279						1	45	23	2							71
280–289						–	24	21	4							49
290–299						–	3	17	8	3						31
300–309						1	2	6	11	2						22
310–319								2	7	–		1				10
320–329								–	4	1	3	2				10
330–339								1	1	–	–	1	2			5
340–349										1	1	1	2	3		8
350–359											1	–		1	1	3
360–369												1		2	1	4
370–379															1	1
380–389															–	0
390–399															1	1
Total	205	9	42	57	106	83	146	89	38	7	5	6	4	6	4	807
%	25.4	1.1	5.2	7.0	13.1	10.2	18.0	11.0	4.7	0.8	0.6	0.7	0.4	0.7	0.4	100.0
Mean length	40.0	93.0	135.0	187.8	209.9	238.9	268.5	281.3	301.3	311.0	333.6	335.7	341.5	354.3	373.5	253.7
95% C.I.[a]	39.3–40.7	87.7–98.3	129.5–140.5	185.1–190.5	207.2–212.6	233.2–244.6	266.1–270.9	278.0–284.6	296.2–306.4	293.6–328.4	317.3–349.9	317.8–355.6	333.3–349.7	345.5–363.1	350.3–396.7	

[a]Confidence interval.

TABLE 2. Mean calculated fork lengths (*mm*) of 586 round whitefish at end of each year of life (sexes combined), Leaf River, 1968.

Age-group	No. fish	Mean length at capture	Year													
			1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	11	92.5	70.2													
2	46	140.0	73.6	104.4												
3	57	188.2	70.2	109.4	155.5											
4	106	209.9	70.2	98.0	147.6	187.1										
5	80	239.5	69.8	98.5	137.2	181.0	217.6									
6	142	267.8	69.2	99.8	141.3	179.1	220.3	250.8								
7	85	281.7	69.1	103.0	143.8	183.4	216.5	248.7	270.0							
8	36	301.8	70.2	100.6	143.8	185.9	219.0	247.6	272.0	289.6						
9	8	312.3	70.3	96.7	140.9	183.7	223.1	249.6	269.2	286.7	299.7					
10	4	336.8	68.0	98.1	141.8	185.8	228.0	259.3	282.0	300.2	318.3	329.9				
11	6	335.7	70.6	100.6	142.9	179.7	223.1	255.1	278.8	296.7	310.8	321.6	331.3			
12	2	340.5	72.3	97.7	143.8	183.1	219.1	253.2	274.8	292.5	306.9	318.9	329.0	338.1		
13	6	354.3	73.9	108.4	149.3	184.8	216.8	250.0	269.6	290.3	308.1	319.8	330.9	342.1	349.9	
14	4	373.5	70.8	105.4	147.1	180.7	218.3	253.0	279.8	297.0	314.7	331.4	341.9	351.4	354.6	369.4
Mean length			70.0	101.2	144.1	182.7	218.8	250.0	271.4	291.1	308.6	324.1	333.3	344.5	354.6	369.4
Annual increment			70.0	31.1	43.0	38.5	36.2	31.2	21.4	19.7	17.4	15.6	9.1	11.2	10.1	14.7

At about 36 mm in length, the fry appear to accelerate their rate of increase in weight relative to length.

No significant difference ($P = 0.05$) was noted between the sexes in weight at a given length where an adequate sample was available. Females age 6 and older tended to weigh more than males, but only at age 6 was the difference significant ($P = 0.05$).

Age and length at maturity and sex ratio — The youngest mature round whitefish of either sex were in their fourth year of life, and all were mature by age 6, with the exception of one male fish at age 7 (Fig. 2). Within an age-group, there were generally similar percentages of mature and immature fish of both sexes, although at ages 4–5 the proportion of mature fish increased more rapidly for females than for males. The shortest mature males were between 210 and 219 mm, and all were mature at 250–259 mm (with one exception); for females the corresponding lengths were 190–199 mm and 240–249 mm, respectively.

Of the 593 specimens for which sex was determined, 40.6% were males. Females outnumbered males in all age-groups where the sample size was large. Males were only slightly less frequent than females up to age 4 but, as the age increased, so did the proportion of females. Of 32 fish age 9 and older, 23 were females.

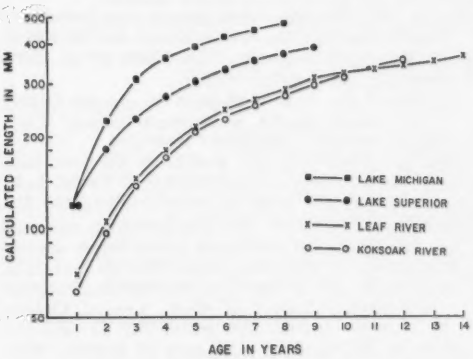

FIG. 1. Calculated lengths at different ages of round whitefish (*Prosopium cylindraceum*) from Ungava and the Great Lakes.

Spawning frequency — Of 147 mature females, 84.3% were ripening spawners. Of the 26 nonspawners, seven appeared to have undergone some development, then regressed. Spawning is apparently not an annual event for all mature fish.

Discussion — The results of this study generally support or extend the conclusions reached by Mackay and Power (1968) concerning round whitefish from Ungava. Ungava round whitefish have a slower growth rate, are older at maturity, and attain a smaller maximum size and weight than do Great Lakes populations. However, the separation of environmental effects on their life history attributable to latitudinal differences and river versus lake habitats is not presently possible.

The extensive overlap in empirical lengths between age-groups appears typical for the species wherever it is found (Bailey 1963; Mraz 1964; Mackay and

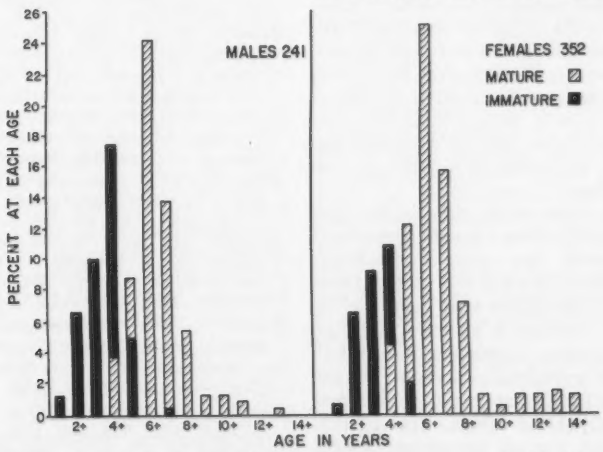

FIG. 2. Age distribution of mature and immature round whitefish, by sex.

Power 1968). The normal distribution of the catch curve for length for this unexploited northern population is in agreement with Johnson's (1972) hypothesis that such population structures are characteristic of northern regions and may be likened to a forest climax condition.

The time of annulus completion is interesting in that, if the period of annulus formation was 3–4 weeks (Hogman 1968) or less, then the initiation of annulus growth closely coincided with the time of ice breakup in the river and subsequent rising water temperature.

The body–scale intercept values for Leaf River round whitefish is greater than those calculated by Bailey (1963) and Mackay and Power (1968), which are similar. It might be expected that the intercept values for Ungava populations would agree more closely between themselves than with the Great Lakes value. A relatively small sample size, with a restricted range of fish lengths such as used by Mackay and Power (1968), might make it difficult to obtain a realistic body-scale equation. Alternatively, the two Ungava populations may actually have dissimilar lengths at the beginning of scale formation.

Assuming that scale growth reflects body growth (Van Oosten 1929), the growing season for round whitefish in the Leaf River is estimated to extend from about mid-June to about mid-September, with the greatest growth occurring in July. Bailey (1963) suggests that the growing season for round whitefish in Lake Superior is at least from the beginning of June to late October.

Leaf and Koksoak River round whitefish decrease in growth rate with age as do more southern populations (Mackay and Power 1968; Bailey 1963; Mraz 1964), but only the Ungava populations showed the atypical decrease in second year growth. This decrease was present in less marked form in the Koksoak River populations, but Mackay and Power (1968) make no note of it. Nebish Lake rock bass (*Ambloplites rupestris*) also showed a second year decrease in growth increment (Hile 1941), but no explanation was offered.

The sexes matured at similar ages in the Leaf River, while Koksoak River females reportedly matured a year earlier than males although this result may be in error (Mackay and Power 1968). All females of both populations were mature at the same age and, also, matured at a smaller size than did males. Why Ungava females mature before or at the same age as males is uncertain; usually males mature before females, as they do in the Great Lakes (Bailey 1963; Mraz 1964). The relation between maturity, size, and age are similar for Leaf and Koksoak River populations and, while the age at maturity is higher for Ungava populations, they reach maturity at a size similar to that of the Lake Superior populations.

The sex ratios of Leaf and Koksoak River fish are identical (41% male). Females outnumber males in virtually every age-group, which is quite unlike the Great Lakes situation.

The reason for late summer gonadal regression in some mature, ripening females is unknown, but it is common for northern freshwater fish not to spawn annually (Kennedy 1953; Vladykov 1956).

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BAILEY, M. M. 1963. Age, growth and maturity of round whitefish of the Apostle Islands and Isle Royale regions, Lake Superior. U.S. Fish. Wildl. Serv. Fish. Bull. 63: 63–75.

GERKING, S. D. 1966. Length of the growing seasons of the bluegill sunfish in northern Indiana. Verh. Internat. Verein. Limnol. 16: 1056–1064.

HILE, R. 1941. Age and growth of the rock bass, *Ambloplites rupestris* (Rafinesque), in Nebish Lake Wis. Trans. Wis. Acad. Sci. Arts Letters 33: 189–337.

HOGMAN, W. J. 1968. Annulus formation on scales of four species of coregonids reared under artificial conditions. J. Fish. Res. Board Can. 25: 2111–2122.

JESSOP, B. M. 1972. Aging round whitefish (*Prosopium cylindraceum*) of the Leaf River, Ungava, Quebec, by otoliths. J. Fish. Res. Board Can. 29: 452–454.

JESSOP, B. M., R. L. G. LEE, AND G. POWER. 1971. Observations on the fish fauna of the Leaf River, Ungava. Can. Field Natur. 84: 365–367.

JOHNSON, L. 1972. Keller Lake: characteristics of a culturally unstressed salmonid community. J. Fish. Res. Board Can. 29: 731–740.

KENNEDY, W. A. 1953. Growth, maturity, fecundity and mortality in the relatively unexploited whitefish (*Coregonus clupeaformis*) of Great Slave Lake. J. Fish. Res. Board Can. 10: 413–441.

MACKAY, I., AND G. POWER. 1968. Age and growth of round whitefish (*Prosopium cylindraceum*) from Ungava. J. Fish. Res. Board Can. 25: 657–666.

MRAZ, D. 1964. Age and growth of the round whitefish (*Prosopium cylindraceum*) in Lake Michigan. Trans. Amer. Fish. Soc. 93: 46–53.

NORDENG, H. 1961. On the biology of char (*Salmo alpinus* L.) in Salangen, north Norway. I. Age and spawning frequency determined from scales and otoliths. Nytt. Mag. Zool. 10: 67–123.

POWER, G. 1964. A technique for preparing scale smears. Trans. Amer. Fish. Soc. 93: 201–202.

VAN OOSTEN, J. 1929. Life history of the lake herring (*Leucichthys artedi* Le Sueur) of Lake Huron as revealed by its scales, with a critique of the scale method. Bull. U.S. Bur. Fish. 44(1928): 265–428.

VLADYKOV, V. D. 1956. Fecundity of wild speckled trout (*Salvelinus fontinalis*) in Quebec lakes. J. Fish. Res. Board Can. 13: 799–841.

Extension of Shelf-Life of Indian Mackerel (*Rastrelliger kanagurta*) by Irradiation

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A dose of 150 krad was optimum for extending refrigerated (0–2 C) shelf-life of fresh Indian mackerel (*Rastrelliger kanagurta*). This dose extended the shelf-life to 25–30 days from 10–12 days without inducing undesirable changes in the organoleptic qualities. Vacuum-packaging affected the appearance of the irradiated product by drawing out muscle fluids during storage. Samples irradiated aerobically did not become rancid and were acceptable to a taste panel. Storage of fish in ice beyond 5 days before irradiation reduced its post-irradiation shelf-life.

VENUGOPAL, V., K. A. SAVAGAON, U. S. KUMTA, AND A. SREENIVASAN. 1973. Extension of shelf-life of Indian mackerel (*Rastrelliger kanagurta*) by irradiation. J. Fish. Res. Board Can. 30: 305–309.

La dose optimale d'irradiation permettant de prolonger la durée de conservation sous réfrigération (0–2 C) du maquereau indien (*Rastrelliger kanagurta*) frais est de 150 krad. Une telle dose assure une durée de conservation de 25 à 30 jours au lieu de 10 à 12 jours, sans causer de changements indésirables des qualités organoleptiques du produit. L'emballage sous vide change l'apparence du produit en retirant les fluides du muscle durant l'entreposage. Les échantillons soumis à une irradiation anaérobie ne deviennent pas rancides et sont jugés acceptables par un groupe de dégustation. L'entreposage du poisson dans la glace durant plus de cinq jours avant l'irradiation diminue sa durée de conservation après irradiation.

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PASTEURIZATION doses of gamma radiation have been used to extend the useful shelf-life of varieties of fishery products at low temperatures (Ronsivalli and Carver 1968; Kumta and Sreenivasan 1970). Radiation response of each species of fish, however, is influenced by inherent differences in their biochemical composition since the chemical constituents are affected in several ways by irradiation (King et al. 1971). Lipids are reported to have a pronounced influence on acceptability of petrale sole, halibut, and swordfish upon irradiation owing to development of rancidity (Slavin et al. 1966). The critical factor governing the radiation response, however, seems to be the composition of the lipids rather than the amount since Atlantic mackerel (*Scomber scombrus*), containing normally 8–22% fat (Ackman and Eaton 1971), responded favorably to irradiation (Slavin et al. 1966). In this respect Indian mackerel (*Rastrelliger kanagurta*), analysing to 1.2–8.2% fat (Venkatraman and Chari 1950) and possibly differing in its lipid composition from that of Atlantic mackerel, was investigated for its amenability to irradiation.

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This report concerns effects of various doses of gamma irradiation on total bacterial count and acceptable shelf-life of Indian mackerel, influence of air- and vacuum-packaging on its organoleptic acceptability and oxidative changes, and effects of pre-irradiation storage time on the post-irradiation shelf-life.

Materials and methods — Fresh mackerel caught at the Bombay shore were brought to the laboratory in ice immediately on landing. The fish were washed clean in potable water, eviscerated, and beheaded. The dressed fish were washed again, drained, and wrapped in cellophane film. The wrapped fish were sealed in polyethylene bags without and under vacuum; prior wrapping helped to keep pouches dry and facilitated vacuumizing and sealing.

To evaluate the effect of ice storage of mackerel before irradiation on the post-irradiation shelf-life, the fish were held in ice for different periods of time prior to irradiation.

Some of the fish were also treated with common salt for 1 hr to prevent drip formation in vacuum-packed fish. Fish to salt ratio was 1:1. The treated fish were washed well, wrapped in cellophane film, and packed under vacuum.

The packaged fish were irradiated in ice at doses ranging from 0 to 200 krad (Table 1) in a Cobalt-60 Package

TABLE 1. Initial TBA values[a] and organoleptic scores[a] of mackerel irradiated at various doses and shelf-lives of samples at 0–2 C.

Dose (*krad*)	TBA value (μg malonaldehyde/g)	Organoleptic score	Shelf-life days[b]
0	1.0	7.7	10–12
50	1.9	7.0	14–15
100	1.8	6.6	18–20
150	1.8	6.6	25–28
200	1.8	6.0	25–28

[a]Values based on 2 tests.
[b]Based on mean organoleptic score of 5 or higher.

Irradiator (Atomic Energy of Canada Ltd.) at 360 krad per hour. Unirradiated and irradiated samples were stored in ice till taken for quality evaluation.

The total aerobic viable microbial counts in the homogenate prepared from two fish for each treatment were determined by the standard pour-plate method using tryptone–glucose–yeast extract agar medium. Plates were incubated at 30 C for 2 days before the colonies were counted.

Total volatile basic nitrogen (TVBN) was estimated by Conway microdiffusion technique (Farber and Ferro 1956) using 10% trichloroacetic acid (TCA) extract of two fish from each treatment.

Thiobarbituric acid (TBA) values were estimated according to the method of Sinnhuber and Yu (1958) using 1:5 aqueous extract of one fish centrifuged at 5000 r.p.m. for 10 min. Peroxide value was estimated by the method of Wagner et al. (1947) using chloroform–methanol extract (Bligh and Dyer 1959) of one fish.

Shelf-life was determined using organoleptic acceptability as the main criterion since objective quality values have ultimately to be correlated with it. Organoleptic evaluation was done by a six member panel of experienced tasters using a 9-point hedonic scale. The fish were served after cooking in the pouches by immersing in boiling water for 20 min. Samples were rejected when the mean score was less than 5.

Effects on objective quality values — Reduction in bacterial counts in mackerel on irradiation was nearly logarithmic and the dose required to effect one log cycle kill was of the order of 125 krad (Fig. 1). This value is higher than that (100 krad) for a reduction of between 1 and 2 log orders for cod (Ronsivalli et al. 1970) and haddock (Laycock and Regier 1970) microflora. This could be due to the association with Indian mackerel of relatively radio-resistant bacteria like *Micrococcus*, *Bacillus*, and *Achromobacter* (Venkatraman and Sreenivasan 1952) as compared to *Pseudomonas*, which along with *Achromobacter*, predominate in the bacterial composition of cod (Shewan 1962) and haddock (Laycock and Regier 1970).

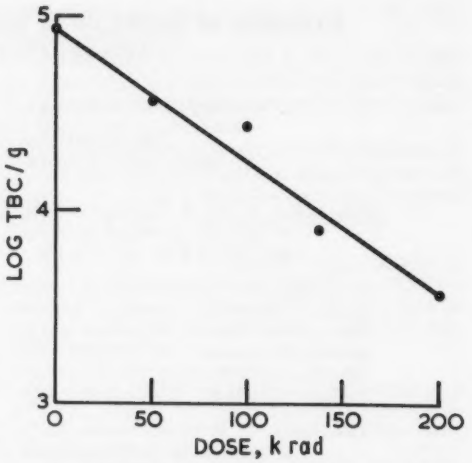

FIG. 1. Total bacterial counts of mackerel after irradiation at various doses. Values are based on two tests.

The effectiveness of 150 krad was much greater than that of 100 krad and similar to that of 200 krad concerning the growth of surviving flora in vacuum-packed irradiated fish (Fig. 2).

A dose of 150 krad was more effective than 100 krad and as effective as 200 krad in retarding the formation of TVBN substances during subsequent ice storage of vacuum-packed fish (Fig. 2). TBA values for samples irradiated with up to 150 krad increased during the first 5 days of storage and then decreased, whereas those for samples irradiated at 200 krad reached the maximum at 20 days and then declined. The fall in TBA reacting substances after an initial rise during storage, which has also been reported for irradiated pomfret (Kamat and Kumta 1972) and for cooked, irradiated shrimp (Savagaon et al. 1972), could be due to their highly reactive properties leading to interaction with themselves and with proteins and amino acids. Also, polyethylene being permeable to gases, some of the volatile TBA reducing substances may have escaped during storage.

Optimum dose and shelf-life — On irradiation with up to 150 krad, samples showed no detectable changes in odor or flavor, the scores for acceptability being 6.6–7.7 (Table 1). At 200 krad, with the presence of slight objectionable odors and flavors, the score was 6. Rancid type odors and flavors were not noticed at any time in any of the irradiated samples, in spite of the increase

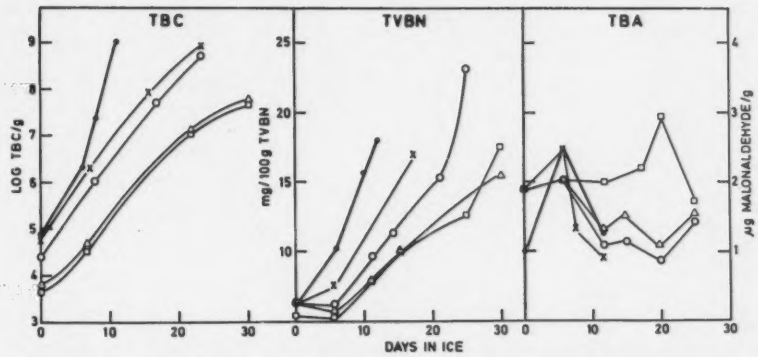

FIG. 2. Total bacterial counts, total volatile basic nitrogen, and thiobarbituric acid values during storage in ice for vacuum-packed mackerel after irradiation at various doses. Values are based on two tests. ●, 0 krad; x, 50; ○, 100; △, 150; and □, 200.

in TBA values; lack of correlation between TBA number and sensory changes has also been reported by Spinelli et al. (1969). The shelf-life obtained at 150 krad (25–28 days) was optimum, being considerably more than that at lower doses and equal to that at 200 krad.

The shelf-life of fresh Indian mackerel in ice was longer (10–12 days, Table 1) than for tropical shrimp and Bombay duck, which spoil in 7 days (Kumta and Sreenivasan 1970). This could be due to the predominance of mesophilic organisms in mackerel (Velankar and Kamasastri 1956). Extension in shelf-life obtained by irradiation, however, was of the same order as that for irradiated Bombay duck but longer than that for irradiated shrimp. Termination of storage life of unirradiated and irradiated fish was accompanied with high TBC and TVBN (Fig. 2). While the spoiled unirradiated samples had predominantly putrid odors, their irradiated counterparts had a 'sweet' type of odor.

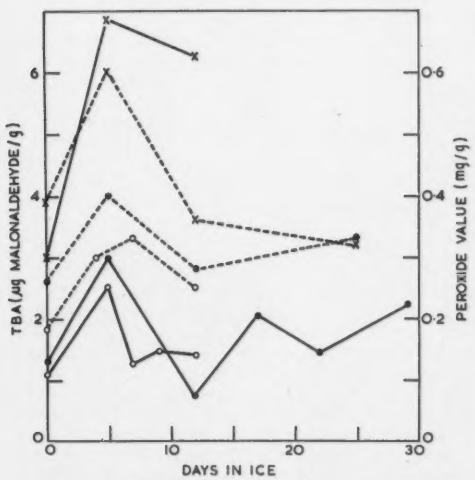

FIG. 3. TBA (——) and peroxide (– – –) values during storage in ice for air- (x) and vacuum-packed (●) mackerel after irradiation at 150 krad, along with values for unirradiated, vacuum-packed samples (○).

Air-and vacuum-packaging — After irradiation at 150 krad, the TBA and peroxide values were higher for air-packed than for vacuum-packed samples (Fig. 3). After prolonged storage, the peroxide values for vacuum- and air-packed samples were similar. TBA values for both packs declined after 5 days storage.

Organoleptic evaluation indicated that air and vacuum-packed samples irradiated at 150 krad were acceptable for 26 and 29 days, respectively (Fig. 4). For the major part of the storage life, quality of the

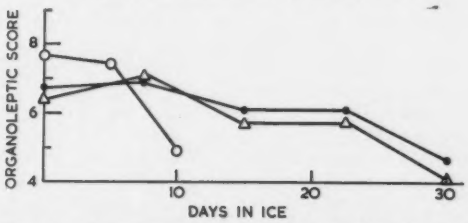

FIG. 4. Organoleptic scores on 9 point hedonic scale during storage in ice for mackerel irradiated at 150 krad in vacuum (●) and in air (△) and for unirradiated vacuum-packed samples (○). Results are based on two tests.

TABLE 2. Total bacterial counts, total volatile basic nitrogen, thiobarbituric acid values, and shelf-lives of one lot of mackerel stored in ice for different periods before irradiation at 150 krad.

Days of storage in ice	TBC/g			TVBN mg/100 g			TBA μg MA[d]/g			Post-irradiation shelf-life (*days*)
	A[a]	B[b]	C[c]	A	B	C	A	B	C	
0	1.3×10^4	1.6×10^3	2.1×10^6	6.11	6.93	8.12	1.06	1.85	1.13	25–28
3	1.6×10^5	1.7×10^4	6.6×10^5	5.84	6.45	7.53	1.90	2.49	1.85	22–25
5	1.4×10^6	9.0×10^4	2.4×10^5	6.81	6.73	8.82	2.40	1.53	1.14	20–23
7	8.7×10^6	1.7×10^5	1.1×10^6	9.32	9.54	12.31	1.21	1.30	1.20	14–15
9	1.2×10^7	4.1×10^5	1.3×10^6	13.41	13.51	18.71	1.45	1.21	1.21	12–13

[a]A, Unirradiated samples analysed on various days during storage.
[b]B, Samples irradiated on various days of ice-storage and analysed immediately.
[c]C, Samples irradiated on various days of ice-storage and analysed on 15th day of storage commencing from the beginning of the test.
[d]MA = malonaldehyde

vacuum-packed irradiated mackerel remained good with acceptability score slightly above 6. Quality of the air-packed irradiated samples during the same period was moderately good with scores around 5.8.

Vacuum-packaging caused drip collection in the pouches and thus affected the overall appearance of the packaged product adversely. Treatment of fish with salt prior to vacuum packaging reduced the drip formation but increased TBA reacting substances in the product (3.7 μg MA/g). In contrast, the unsalted air-packed mackerel accumulated no drip in the pouches and had better appearance. From these results, it was concluded that air-packaging of irradiated fresh mackerel was satisfactory.

Effects of storage in ice before irradiation — The duration of storage of the fish in ice before irradiation had a marked influence on the post-irradiation shelf-life of the product (Table 2). The TBC and TVBN values increased with the period of storage in ice. Organoleptic evaluation of the product showed that the longer the period of ice storage, the shorter was the post-irradiation shelf-life. However, storage in ice up to 5 days gave a post-irradiation shelf-life close to that of freshly irradiated mackerel. Similarly, Ampola and Ronsivalli (1969) have observed that the shelf-life of haddock irradiated after icing for 7–9 days was approximately the same as that for freshly irradiated haddock fillets. In normal commercial practices, fishing vessels return from sea after 2–5 days of continuous fishing. The fish in such cases may be advantageously irradiated to get a shelf-life extension nearly equal to that obtained by irradiating freshly caught fish.

The irradiation process resulted in two to three-fold increases in the shelf-life of Indian mackerel and this could provide sufficient time for distribution of the fish in the fresh state to marketing centers at long distances from the coast.

ACKMAN, R. G., AND C. A. EATON. 1971. Mackerel lipids and fatty acids. Can. Inst. Food Technol. J. 4: 169–174.

AMPOLA, V. G., AND L. J. RONSIVALLI. 1969. Effect of pre-irradiation quality of eviscerated haddock on post-irradiation shelf-life of fillets. J. Food Sci. 34: 27–30.

BLIGH, E. G., AND W. J. DYER. 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. 37: 911–917.

FARBER, L., AND M. FERRO. 1956. Volatile reducing substances and volatile nitrogen compounds in relation to spoilage in canned fish. Food Technol. 10: 303–304.

KAMAT, S. V., AND U. S. KUMTA. 1972. Studies on radiation pasteurization of medium fatty fish. I. Control of radiation induced oxidative changes in white pomfret (*Stromateus cinereus*) by vacuum packa g-ing. Fish. Technol. (India) 9(1): 8–16.

KING, F. J., J. M. MENDELSOHN, D. F. GADBOIS, AND J. B. BERNSTEINAS. 1971. Some chemical changes in irradiated sea foods. *In* Recent advances in the chemistry of food irradiation. Symp. Nat. Meet. Amer. Chem. Soc. Los Angeles, Calif. March 29–April 2.

KUMTA, U. S., AND A. SREENIVASAN. 1970. Preservation by gamma irradiation of Bombay duck, shrimps and white pomfrets, p. 75–104. *In* Preservation of fish by irradiation. Proc. FAO/IAEA Panel, Vienna 1969.

LAYCOCK, A., AND L. W. REGIER. 1970. The effect of gamma irradiation on the microbial spoilage patterns on fish, in relation to initial quality, p. 13–25. *In* Preservation of fish by irradiation. Proc. FAO/IAEA Panel, Vienna 1969.

RONSIVALLI, L. J., AND J. H. CARVER. 1968. Study of irradiated-pasteurized fishery products. Isotop. Radiat. Technol. 5: 224–233.

RONSIVALLI, L. J., J. D. KAYLOR, E. J. MURPHY, R. J. LEARSON, AND M. S. SCHWARTZ. 1970. Studies in petition-oriented aspects of radiation pasteurization of fishery products, p. 1–11. *In* Preservation of fish by irradiation. Proc. FAO/IAEA Panel, Vienna 1969.

SAVAGAON, K. A., V. VENUGOPAL, S. V. KAMAT, U. S. KUMTA, AND A. SREENIVASAN. 1972. Radiation preservation of tropical shrimp for ambient temperature storage. 2. Storage studies. J. Food Sci. 37: 151–153.

SHEWAN. 1962. The bacteriology of fresh and spoiling fish and some related chemical changes, p. 167–193. *In* J. Hawthorn and J. Muil Leitch [ed.] Recent advances in food science. Butterworth & Co. (Publishers) Limited, London.

SINNHUBER, R. O., AND T. C. YU. 1958. 2-thiobarbituric acid method for the measurement of rancidity in fishery products. II. The quantitative determination of malonaldehyde. Food Technol. 12: 9–12.

SLAVIN, J. W., J. T. R. NICKERSON, S. A. GOLDBLITH, L. J. RONSIVALLI, J. D. KAYLOR, AND J. J. LICCIARDELLO. 1966. The quality and wholesomeness of radiation pasteurized marine products with particular reference to fish fillets. Food Irradiat. 3: 365–381.

SPINELLI, J., G. PELROY, AND D. MIYAUCHI. 1969. Quality indices that can be used to assess irradiated sea foods, p. 425. *In* R. Kreuzer [ed.] Freezing and irradiation of fish. Fishing News (Books) Ltd., London.

VELANKAR, N. K., AND P. V. KAMASASTRI. 1956. The bacterial flora, trimethylamine and total volatile nitrogen of fish muscle at 0°C (in ice). Indian J. Fish. 3: 269–289.

VENKATRAMAN, R., AND S. T. CHARI. 1950. Studies on mackerel fat variation — correlation of plankton fat with fat of fish. Proc. Indian Acad. Sci. Sect. B 37(6): 224–227.

VENKATRAMAN, R., AND A. SREENIVASAN. 1952. A preliminary investigation on the microflora of the mackerels of the west coast. Indian J. Med. Res. 40: 529–533.

WAGNER, C. D., H. L. CLEVER, AND E. D. PETERS. 1947. Determination of organic peroxides, evaluation of ferrous thiocyanate colorimetric method. Anal. Chem. 19: 980–982.

A Comparison of Benthic Microalgal Production Measured by C^{14} and Oxygen Methods

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HUNDING, C., AND B. T. HARGRAVE. 1973. A comparison of benthic microalgal production measured by C^{14} and oxygen methods. J. Fish. Res. Board Can. 30: 309–312.

A comparison of estimates of benthic primary production on a sandy beach measured by in situ oxygen and laboratory C^{14} methods showed that both methods gave similar measures of the magnitude of production. Sources of error in each method are discussed. Measures of C^{14} uptake offer sensitivity when production is low, but when undisturbed sediment cores can be obtained, production is most easily measured by following changes in dissolved oxygen.

HUNDING, C., AND B. T. HARGRAVE. 1973. A comparison of benthic microalgal production measured by C^{14} and oxygen methods. J. Fish. Res. Board Can. 30: 309–312.

Une comparaison des estimés de la production primaire du benthos d'une plage sablonneuse déterminée par mesure d'oxygène in situ et de C^{14} au laboratoire démontre que les deux méthodes donne des résultats semblables. Nous analysons les sources d'erreur inhérentes aux deux méthodes. Les mesures de captation du C^{14} offrent un haut degré de sensibilité lorsque la productivité est faible. Cependant, lorsqu'on peut obtenir une carotte de sédiments qui n'ont pas été remués, il est plus facile de mesurer la productivité par l'étude des variations de l'oxygène dissous.

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CHANGES in dissolved oxygen concentrations over light and dark undisturbed sediment cores have been used to estimate in situ primary production by benthic algae (Pomeroy 1959; Pamatmat 1968; Hargrave 1969). This method, where organisms are undisturbed, contrasts techniques using C^{14} where surface sediment is removed from a core and incubated under various light conditions in the laboratory and actual production is calculated from this measure of potential production (Grøntved 1960; Gargas 1970; Hunding 1971). Baird and Wetzel (1968) incubated resettled sand with C^{14} in situ and described a method for calculating production of attached flora on sand grains.

A comparison of estimates of benthic primary production measured by oxygen and C^{14} methods with samples from the same location would be useful. Both methods are in common use (Pamatmat 1968) but different assumptions are involved in calculating production by each method and experimental artifacts introduced by sampling and incubation also differ. While such a comparison cannot account for errors introduced by these assumptions and artifacts, some evaluation of the relative measures of benthic primary production by the two methods can be made. This is attempted in the present study.

Methods — Samples were taken from an exposed sandy beach at the north end of Lake Esrom, Denmark (Berg 1938; Jonasson 1965). Water (30–40 cm deep) covered the sand that was usually well sorted with a mean grain diameter of 0.2–0.3 mm. Sparse patches of *Potamogeton perfoliatus* with filaments of green algae, mainly *Cladophora* sp., occurred in spring and early summer. On windy days the beach was exposed to strong wave action and surface sand was mixed to at least a depth of 1 cm.

Changes in dissolved oxygen over sand cores (20 cm^2) were followed in situ and net production was calculated as described by Hargrave (1969). Measurements of sediment core oxygen uptake, made in the laboratory, permitted an estimate of gross production. Phytoplankton oxygen production and respiration in water over sediment was insignificant in comparison to changes occurring over sediment and no correction was made. A P.Q. of 1.25 and R.Q. of 0.85 was assumed in calculating gross carbon production from changes in dissolved oxygen (Strickland and Parsons 1960). While these values are to some degree arbitrary, they are reasonable estimates for comparative purposes.

C^{14} uptake by algae on surface sand grains was determined as described by Hunding (1971). The method, first developed by Grøntved (1960) using surface sand to 1-cm depth, was altered to take only the upper 3 mm of sand cores. This depth includes only sand from the photic zone of the sediment (Gargas 1970; Hunding 1971). Only algae firmly attached to sand grains are included by the sampling procedure.

Potential production, measured by C^{14} uptake, is converted to an estimate of actual production by a conversion factor derived from calculated light penetration into the sediment, measured light intensity and knowledge of photosynthesis–light curves for samples taken during the year (Hunding 1971). Since we intended to compare primary production measured by oxygen and C^{14} methods on an in situ basis, total radiation reaching the sediment during an experiment was estimated. A recording phyrheliometer and an underwater photometer (selenium photoelectric cell) with opal and neutral filters (adsorption 3500–7000 $m\mu$) was used for this purpose.

Results and discussion — Fourteen experiments were performed at various times between September 1970 and July 1971 (Table 1). Potential production, uncorrected for in situ light conditions, was always much greater than gross production actually measured in situ by oxygen methods except during November. Actual production values calculated from C^{14} uptake also exceeded rates determined by the oxygen method in 10 out of 14 measurements, although the discrepancy is small in many cases. Deviation of production estimates by oxygen and C^{14} methods during June was 14 and 18% with four replicate samples.

Differences in production estimates calculated by the two methods were not related to temperature since deviation was equally great in fall, winter, and summer. Light conditions during field measurements, however, were related to differences in estimates of production by the two methods. When light intensity was high, the oxygen method gave lower estimates of production than that calculated from C^{14} uptake. On overcast days and in late autumn, when light intensity was low, measures of production by the C^{14} method were lower.

Discrepancies between measurements of benthic microalgal production by C^{14} and oxygen methods in the first 10 experiments are not important if the data were intended to indicate the approximate magnitude of production. Both methods show similar changes in successive measures of production. Deviations in the last series of experiments, however, are so great as to make both methods suspect.

Several sources of error may be involved in these discrepancies. Bubble formation often occurred in light-exposed sediment cores. Water covering the sediment before in situ experiments began was always saturated or supersaturated with oxygen as a result of waves and water movement onto the beach. Subsequent exposure of sand cores to light, even for periods as short as 1.5 hr, resulted in further increases in dissolved oxygen concentration. Small bubbles often formed in the sand and on cylinder walls. Measures of dissolved oxygen in water over

TABLE 1. Microalgal production on a Lake Esrom sandy beach at various times of the year. Production estimates by the oxygen method are means of replicate sediment cores and by the C^{14} method are calculated potential production from C^{14} uptake by at least three subsamples of sand grains from the upper 3 mm of one sediment core.

Date 1970/71	Temp. (C)	Mean light at sediment during experiment (μ-amps)	mg C m^{-2} hr^{-1}		
			Oxygen	C^{14}	
				Potential	Actual
Sept. 8	16.0	75	36	102	31
16	15.5	106	49	178	78
24	13.1	150	50	119	64
Oct. 12	12.6	42	67	215	75
22	9.2	95	27	130	66
29	8.5	80	21	55	25
Nov. 5	8.3	10	8	16	2
12	8.0	33	6	9	3
26	5.8	8	4	3	1
Apr. 28	6.2	130	21	49	26
June 21	15.0	135	36	113	47
28	14.8	170	56	151	74
July 12	19.3	75	50	215	69
19	14.5	73	31	247	79

the sediment did not include oxygen in this form. This error, most critical on sunny days with maximum oxygen evolution, would result in an underestimate of production. Although short-term incubations were used under these conditions to minimize the error, this might explain why C^{14} measured rates of production are much higher than rates determined by the oxygen method on days of high light intensity.

Calculations of carbon production from oxygen evolution are based on an average P.Q. of 1.25. This value may vary with light intensity, nutrient conditions and for different species. Eppley and Sloan (1965), for example, observed high P.Q. values (up to 1.9) for marine diatoms which was apparently not associated with nutrient deficiency and independent of excretion. Diatoms may form a large part of the microflora populations in sand (Hunding 1971) and an assumed P.Q. of 1.25 for benthic microalgal communities may be too low. The choice of a higher value would lower calculated rates of production by the oxygen method.

The C^{14} method, despite its sensitivity for measuring carbon uptake, also involves numerous assumptions. C^{14} uptake may represent an increase in cell carbon and thus approximate net production (Eppley and Sloan 1965; Nalewajko 1966) or something between net and gross production may be measured in short-term experiments. Ryther (1956) found close agreement between C^{14} accumulation and net production of *Dunaliella* as measured by the light–dark bottle oxygen method. Earlier experiments, however, suggested that oxygen measurements quantified gross production (Ryther and Vaccaro 1954). However data on C^{14} uptake are interpreted, something less than gross production is probably measured. Other errors aside, production estimates by the C^{14} method should be less than those calculated by following changes in dissolved oxygen over sediment cores. Measures of C^{14} uptake have been increased by 11% to correct for assumed respiration and isotope discrimination Steeman Nielsen 1955). Thus gross potential production has been estimated (Table 1).

Mixing sand grains during incubation with C^{14} might be expected to increase rates of production. In undisturbed sediment algae may only photosynthesize on the surface exposed to light while in laboratory experiments stirring probably exposes greater surface area. Baird and Wetzel (1968) observed a slight decrease in C^{14} uptake with periodic shaking. The error is most likely not serious with surface sand grains from beaches where wave action constantly stirs the sediment.

Probably the most important calculation in converting potential production to estimates of actual production are correction factors for assumed light penetration. Although mixing of sand by wave

action probably ensures homogeneity of microalgal distribution, at least in the upper 3 mm of a wave-washed beach, sorting, geometrical packing, and the shape of the sand grains and grain-size may change temporally and within small sampling areas. Baird and Wetzel (1968) have demonstrated that self-absorption of C^{14} increases as sand grain-size decreases. Thus, these variables may affect C^{14} counting efficiency as well as the conversion of potential to actual production.

Weather conditions during late June and July 1971 were unsettled and overcast skies with wind and rain prevailed. The beach was eroded and sand was displaced as a result of wave action during some of these storms and marked changes in sorting and the abundance of small particles may have occurred. Although this was not quantified, very fine sand was often observed covering coarser grains. Such layering would reduce both light penetration into the sediment and production measured in situ by the oxygen method. C^{14} uptake by stirred surface sand grains would not be affected by these changes.

Conclusion — Neither the oxygen nor C^{14} method is free from experimental artifacts and assumptions that may alter production estimates considerably. Both methods apparently quantify the magnitude of production (Table 1) and in this respect relative changes may be indexed. While the C^{14} method is time-consuming and requires special equipment, it has the advantage that production by attached and free-living algae can be separated and both the light response and vertical distribution of algae in sediment can be considered directly (Hunding 1971). On the other hand, measures of oxygen exchange across an undisturbed sediment surface offer a simple and rapid evaluation of the relative importance of phototrophic and heterotrophic processes under in situ conditions.

For routine determination of the magnitude of benthic primary production in shallow-water sediments from which undisturbed sediment cores can be taken, the oxygen method is easier. Measures of C^{14} uptake, however, offer added sensitivity with sediment from deep water or under low light and temperature conditions where low production would be expected. It is also probably most suitable in well-mixed surface sediments such as wave-washed beaches. Microalgal production in permanently stratified sediment however, is best measured in an undisturbed sediment–water sample by following changes in dissolved oxygen. Chemical gradients and community structure thus remain intact.

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BAIRD, J. E., AND R. G. WETZEL. 1968. A method for determination of zero thickness activity of 14-C labelled benthic diatoms in sand. Limnol. Oceanogr. 13: 379–382.

BERG, K. 1938. Studies on the bottom animals of Esrom Lake. K. Dan. Vidensk. Selsk. Skr. Nat. Math. Afd. 9 Rk. 8: 1–225.

EPPLEY, R. W., AND P. R. SLOAN. 1965. Carbon balance experiments with marine phytoplankton. J. Fish. Res. Board Can. 22: 1083–1097.

GARGAS, E. 1970. Measurements of primary production, dark fixation and vertical distribution of the microbenthic algae in the Øresund. Ophelia 8: 231–253.

GRØNTVED, J. 1960. On the productivity of microbenthos and phytoplankton in some Danish Fjords. Medd. Dan. Fisk. Havunders. 3: 55–91.

HARGRAVE, B. T. 1969. Epibenthic algal production and community respiration in the sediments of Marion Lake. J. Fish. Res. Board Can. 26: 2003–2026.

HUNDING, C. 1971. Production of benthic microalgae in the littoral zone of a eutrophic lake. Oikos 22: 389–397.

JÓNASSON, P. M. 1965. Factors affecting population size of *Chironomus anthracinus* in Lake Esrom. Mitt. Int. Ver. Theor. Angew. Limnol. 13: 139–162.

NALEWAJKO, C. 1966. Photosynthesis and excretion in various planktonic algae. Limnol. Oceanogr. 11: 1–10.

PAMATMAT, M. M. 1968. Ecology and metabolism of a benthic community on an intertidal sandflat. Int. Rev. Hydrobiol. 53: 211–298.

POMEROY, L. R. 1959. Algal productivity in salt marshes of Georgia. Limnol. Oceanogr. 4: 386–397.

RYTHER, J. H. 1956. The measurement of primary production. Limnol. Oceanogr. 1: 72–84.

RYTHER, J. H., AND R. F. VACCARO. 1954. A comparison of the oxygen and ^{14}C methods of measuring marine photosynthesis. J. Cons. Cons. Perma. Int. Explor. Mer. 20: 25–34.

STEEMANN NIELSEN, E. 1955. The interaction of photosynthesis and respiration and its importance for the determination of C-14 discrimination in photosynthesis. Physiol. Plant. 8: 945–953.

STRICKLAND, J. D. H., AND T. R. PARSONS. 1960. A manual of sea water analysis. Fish. Res. Board Can. Bull. 125: 185 p.

A Submersible Spectroradiometer and Data Acquisition System

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DUVAL, W. S., T. J. BROWN, AND G. H. GEEN. 1973. A submersible spectroradiometer and data acquisition system. J. Fish. Res. Board Can. 30: 313–316.

A submersible light meter that measures and records irradiance versus wavelength over the range 400–750 nm is described. A data acquisition system coupled to the radiometer permits collection and processing of large numbers of field observations. The incident spectrum is determined by an optical system incorporating a wedge interference filter monochromator and photodiode. Recorded data are converted to spectral irradiance (μw cm^{-2} nm^{-1}) at 5-nm intervals by applying standard lamp calibration and immersion factor corrections. Minimum detectable radiation varies from 0.05 to 0.016 μw cm^{-2} nm^{-1} depending on wavelength. Response of the meter is not affected by temperature in the range of 3.5–23.0 C and angular dependence of the light collector follows Lambert's Cosine Law for incident angles less than 55° from the vertical.

Changes in the spectral composition of incident light with depth are demonstrated for two lakes.

DUVAL, W. S., T. J. BROWN, AND G. H. GEEN. 1973. A submersible spectroradiometer and data acquisition system. J. Fish. Res. Board Can. 30: 313–316.

Les auteurs décrivent un photomètre submersible capable de mesurer et d'enrégistrer l'irradiation versus la longueur d'onde sur la gamme de 400 à 750 nm. Un système d'acquisition des données couplé au radiomètre permet de cueillir et de traiter un volume considérable d'observations sur le site. Le spectre d'incidence est déterminé par un système optique incorporant un monochromateur à filtre interférentiel et un photodiode. Les données enrégistrées sont converties en irradiation spectrale (μw cm^{-2} nm^{-1}) à des intervalles de 5 nm par calibration standard de lumière et par application de facteurs de correction pour l'immersion. Le minimum de radiation décelable varie de 0.05 à 0.016μw cm^{-2} nm^{-1}, selon la longueur d'onde. La sensibilité du photomètre n'est pas affectée par la température entre 3.5 et 23.0 C, et la dépendance angulaire du collecteur de lumière suit la loi du cosinus de Lambert pour des angles d'incidence de moins de 55° de la verticale.

Les auteurs démontrent les variations de composition spectrale de la lumière incidente en rapport avec la profondeur dans deux lacs.

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VARIOUS workers have described instruments which measure underwater light but the Scripps spectroradiometer, as described by Tyler and Smith (1970), represents one of the current standards in submersible radiometers and utilizes double monochromators with a photomultiplier system to produce spectral irradiance data over 5 log cycles at 5-nm intervals from 362 to 736 nm. The spectroradiometer described in this paper is less sensitive (4 log cycles) but yields spectral irradiance data at 5-nm intervals over a similar wavelength range. The principle advantage of the system described here is the speed of data retrieval; field or lab measurements are collected and processed routinely through a data acquisition system.

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Description — The basic instrument modified for underwater use was an ISCO Model SR Spectroradiometer[1]. The diffusing screen on the light meter (Fig. 1) produces an angular response which follows Lambert's Cosine Law (Fig. 2). Light then passes through a light chopper and monochromator which isolates a narrow bandwidth (15 nm) of light detected by the measuring photodiode. The output of the photodiode is amplified and transferred to an indicating meter through a coherent detector. The Model SR ISCO Spectroradiometer is used in conjunction with a second unit, the Model SRR, which records the spectral irradiance as a continuous distribution from 400 to 750 nm.

In its original form the ISCO was not designed for underwater applications. We enclosed the Model SR

[1]Manufactured by Instrumentation Specialities Co., Inc., 5624 Seward, Lincoln, Nebraska, 68507, U.S.A.

radiometer in an aluminum case (35 cm × 27 cm × 27 cm) with an opal glass collector (10 cm × 10 cm × 6 mm) positioned over the diffusing screen of the light meter. The radiometer is connected to the Model SRR recorder which is kept at the surface, by 30 m of 20 conductor submersible cable.

Originally, the sensitivity adjustment switch was located on the radiometer. However, underwater use required that sensitivity be changed from the surface. This was achieved by installing a pair of 12-position relays on the meter and recorder.

A Datex Model DSR-1[2] data acquisition system was incorporated to minimize the time required to prepare data for computation. Approximately 20 min were required to transfer the readings from one recorder scan (of which there could be more than 200 per day) to computer punch cards. A retransmitting potentiometer converted the zero to full scale pen deflection of the recorder to a 0–100 mv signal which was fed into an API Model 4304 digital voltmeter. This latter device digitized the analog signal to produce a three character digital reading at 5-nm intervals throughout the scan. These data were printed by a teleprinter and punched on 8-level paper tape in ASCII code. Another relay was installed to restart the spectroradiometer after each completed scan and to achieve full synchronization with the timing cycle of the Datex.

Punched cards are prepared from the paper tape by an IBM Model 047 tapereader/keypunch. A computer program was written to permit calculation of the irradiance over each 5-nm segment of the spectrum and the total radiant energy throughout the entire 400–750-nm range. Spectral distributions are plotted by a Calcomp Model 563 off-line plotter facility.

The ISCO Model SR and Model SRR can be powered in the field from a 12 VDC battery. An 80 amp-hr battery will allow about 500 scans. However, the use of the 120 VAC data acquisition system required a 1500-w portable generator as a field power source.

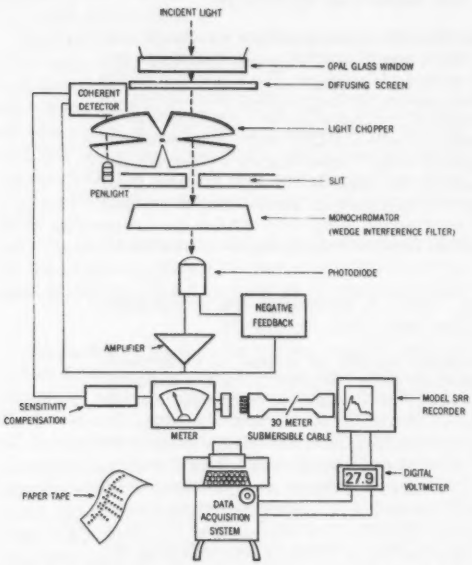

FIG. 1. Simplified block diagram of spectroradiometer/data acquisition system.

Calibration and stray light — The calibration factor, $K(\lambda_i) = H_c(\lambda_i)/G_c(\lambda_i)$, was determined for each wavelength using a table of spectral irradiance,

[2]Manufactured by Datex Division of Conrac Corporation, 1600 S. Mountain Ave., Duarte, California, 91010, U.S.A.

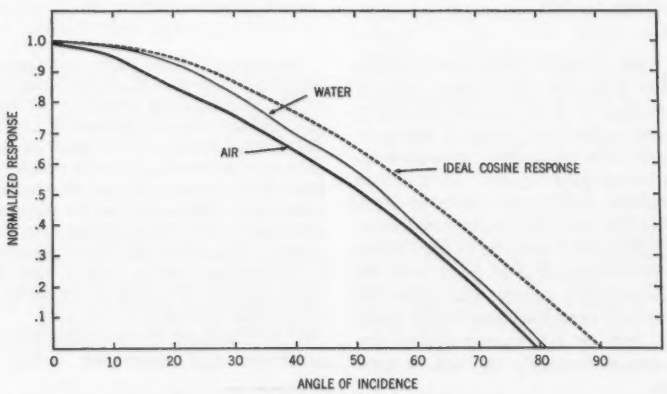

FIG. 2. Angular response of the spectroradiometer light collector in air and water. Shown relative to an ideal response following Lambert's Cosine Law.

H_c (λ_i), supplied with the ISCO Model SRC standard lamp. Before applying calibration factors to the field data, the radiance measurements, $G_c(\lambda_i)$, were corrected for stray light error.

The stray light response was determined by mounting various interference filters over the opal glass collector and measuring the spectral irradiance at wavelengths outside the input bandwidth (Tyler and Smith 1970). Stray light error was less than 0.01% over all wavelengths. Spectral irradiances, $H(\lambda_i)$, were calculated by multiplying recorded data, $G(\lambda_i)$, by the calibration factor, $K(\lambda_i)$, and immersion factor discussed in a subsequent section.

Bench tests — Laboratory tests of temperature dependency, sensitivity, repeatability, cosine response, and immersion error were conducted to assess the reliability and spectral characteristics of the meter.

The manufacturer states that the Model SR Spectroradiometer can be used over a temperature range of 7–43 C. The results of our tests did not support this specification. Temperature fluctuations within the limits of 3.5–23.0 C had no significant effect ($P > 0.01$) on the light response. However, temperatures outside this range significantly lowered the light measuring capacity ($P < 0.01$).

The data acquisition system could not be used at temperatures less than 0 C due to immobilization of the teletype mechanism.

Line voltages of 110–120V at frequencies from 50 to 60 hz are suitable for operation of the spectroradiometer. However, voltages of 110–120V at 59–61 hz are necessary for normal operation of the teletype. A frequency meter and voltmeter are used as a check when operating the system in the field.

The minimum practical sensitivity of the meter is limited by the ability of the digital voltmeter to read the 10V maximum output of the amplifier to 0.1 mv. Since both calibration and immersion factors are wavelength dependent, the minimum detectable intensity is also a function of wavelength. The limit of resolution increases with wavelength from 0.05 μw cm^{-2} nm^{-1} at 400 nm to 0.016 μw cm^{-2} nm^{-1} at 750 nm. In practice, intensities less than 0.5 μw cm^{-2} over the entire visible spectrum cannot be resolved due to random fluctuations in the signal output from the spectroradiometer. On the other hand, the instrument is capable of measuring extremely high intensities; the upper limit is 5.0×10^6 μw cm^{-2} over the visible spectrum. By contrast, natural intensities typically vary from 0.50×10^5 μw cm^{-2} to 1.50×10^5 μw cm^{-2} depending on cloud cover, latitude, and season.

Repeatability or precision of the instrument in replicate scans ($n = 10$) of a standard source is intensity dependent but high in all cases. At 100 μw cm^{-2} repeated measurements agree within 3.4% and at intensities 10 times greater, precision is increased to ±1.5%.

The angular response of the light meter was determined in air and water by rotating the meter about the axis of a directional light source. The angular response of the collector in water was better than in air and approximated a perfect cosine response (Lambert's Cosine Law) for angles less than 55° (Fig. 2). Although the angular response could be improved by redesigning the collector following the specifications of Tyler and Smith (1970), it is adequate for most field and laboratory applications. However, it should be modified if utilization is required where much of the radiation is incident at angles greater than 55° from the vertical.

Since the spectroradiometer was calibrated in air, all data were corrected for the immersion effect arising from glass–water interface phenomena (Tyler and Smith 1970). An immersion factor was calculated for each 5-nm wavelength increment in the spectrum by least squares linear regresison of the ratio of the radiance at a given depth over the

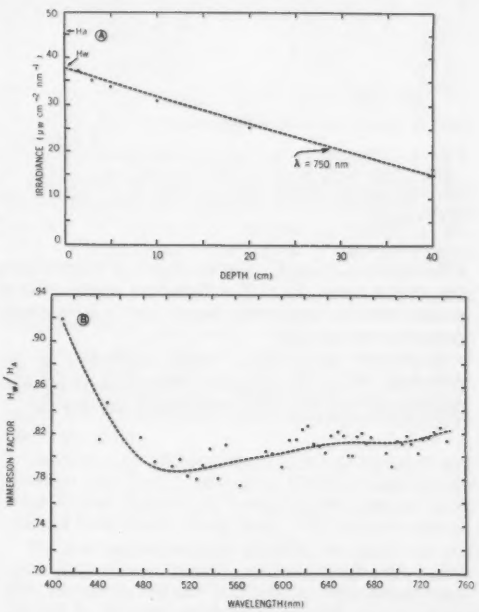

FIG. 3. A, Typical regression of attenuated radiant energy versus depth of water. Used for determination of immersion factor; B, The ratio of the spectroradiometer response in air and water (immersion factor) versus wavelength.

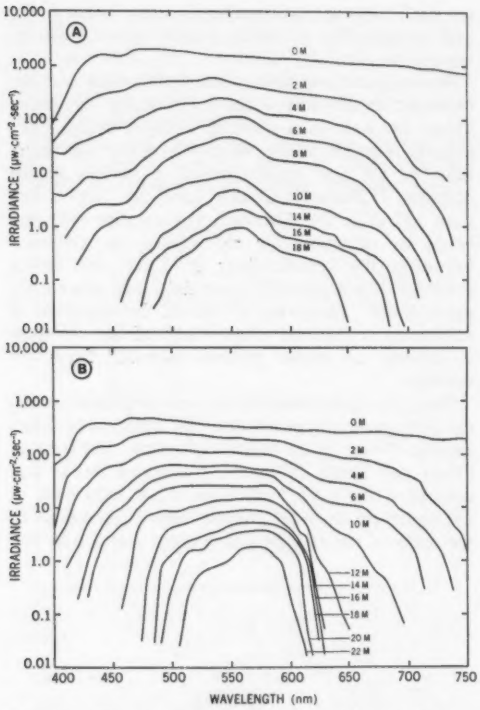

FIG. 4. Changes in the spectral distribution of downwelling light with depth. A, Eunice Lake, B.C., May 27, 1971 (noon); B, Great Central Lake, B.C., August 24, 1971 (noon).

radiance in air (G_W/G_A) versus depth of water above the sensor (Fig. 3A). The intercept of this linear equation is the immersion factor and is wavelength dependent (Fig. 3B).

Radiance data, $G(\lambda_i)$, were converted to irradiance, $H(\lambda_i)$, by applying wavelength dependent calibration factors, and immersion corrections.

Field tests — The submersible spectroradiometer and data acquisition system were used in a variety of marine and freshwater environments. Each scan requires 2.5 min to complete; corrections for changes in the surface intensity during this time can be made by simultaneous recording from a surface radiometer. When wave action is significant, spectral distribution data near the surface show considerable fluctuation and must be corrected by visual approximation. Under normal conditions this is only a significant factor in the first three meters.

The spectroradiometer has been used extensively by one of us (W.S.D.) in studies of zooplankton physiology. Distributions of the spectral irradiance of downwelling light in two lakes, Eunice Lake and Great Central Lake, B.C., are shown in Fig. 4A and B, respectively. Both lakes show rapid differential extinction of red (650–750 nm) and to a lesser extent blue (400–500 nm) light, a characteristic of oligotrophic waters.

These data are typical of the type of measurements that are made routinely with this apparatus. This spectroradiometer/data acquisition system provides a rapid method of quantifying temporal and spatial changes in the light distributions of both marine and freshwater environments.

Acknowledgments — The development of this instrument has been made possible by a National Research Council of Canada Operating Grant to one of us (G.H.G.). Mr H. Dykstra of the Simon Fraser University Electronics Shop contributed to the design of the relay systems. Thanks are due to Mr R. J. Eccles and Mr W. Young, of Allied Scientific Co., for their assistance in developing this instrument and resolving some early difficulties.

TYLER, J. E., AND R. C. SMITH. 1970. Measurements of spectral irradiance underwater. Gordon and Breach Science Publishers, New York, N.Y. 103 p.

Influence of Appearance of Prey and Satiation of Predator on Food Selection by Northern Pike (*Esox lucius*)

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COBLE, D. W. 1973. Influence of appearance of prey and satiation of predator on food selection by northern pike (*Esox lucius*). J. Fish. Res. Board Can. 30: 317–320.

Northern pike (*Esox lucius*) were put in tanks with fantail and regular-tail goldfish (*Carassius auratus*) or with goldfish of different colors. Physical appearance of prey did not affect the pike's feeding. In experiments in plastic pools four species of fish were exposed to predation by northern pike that were satiated or deprived of food for either 2 or 4 weeks. The pike always selected carp (*Cyprinus carpio*) and fathead minnow (*Pimephales promelas*) over green sunfish (*Lepomis cyanellus*) and bluegill (*L. macrochirus*).

COBLE, D. W. 1973. Influence of appearance of prey and satiation of predator on food selection by northern pike (*Esox lucius*). J. Fish. Res. Board Can. 30: 317–320.

Des grands brochets (*Esox lucius*) ont été maintenus en viviers avec des cyprins dorés (*Carassius auratus*) à queue fantaisiste et à queue ordinaire, ou avec des cyprins de différentes couleurs. L'apparence physique de la proie n'a pas eu d'influence sur l'alimentation du brochet. Au cours d'essais en bassins de plastique, nous avons exposé quatre espèces de poissons à la prédation de brochets rassasiés ou privés de nourriture depuis deux ou quatre semaines. Dans tous les cas, le brochet a choisi la carpe (*Cyprinus carpio*) et le méné à grosse tête (*Pimephales promelas*), de préférence au crapet vert (*Lepomis cyanellus*) et au crapet à oreilles bleues (*L. macrochirus*).

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MAUCK and Coble (1971) considered vulnerability of prey to be determined by preference of predator and availability of prey. Further, they stated that preference could be affected by innate inclination, satiation and conditioning of a predator, and availability by appearance and behavior, relative abundance, size, distribution, and armament of prey. Purposes of this study were to determine the effect of physical appearance of prey and satiation of predator on predation by northern pike (*Esox lucius*).

Materials and methods — In two sets of experiments prey fishes were presented to pike in tanks or pools. Goldfish (*Carassius auratus*) with different kinds of tails and of different colors were used in the first experiments. In the second, fathead minnow (*Pimephales promelas*), carp (*Cyprinus carpio*), bluegill (*Lepomis macrochirus*), and green sunfish (*L. cyanellus*)[2] were exposed to pike that had been subjected to various periods of food deprivation.

Appearance of prey — Three experiments were conducted from March 16 to June 7, 1971, in two fiberglass tanks at the University of Missouri in Columbia, Mo. The tanks received a slow flow of charcoal-filtered tap water that ranged in temperature from 10 to 21 C and averaged 14.5 C. The tanks contained 720 liters of water, two air stones, and two shelters. Shelters in one tank were a cement building block standing on end and a tunnel about the same size as the block but constructed of bricks; both shelters in the other tank were brick tunnels. The tanks had a plywood cover and were subjected to a 12-hr light cycle from fluorescent bulbs affixed under the cover.

Goldfish were obtained from a private hatchery and northern pike from a local pond. Kinds of goldfish used were fantail, regular-tail, orange (gold) colored, white colored with small orange flecks, and natural colored.

To start an experiment goldfish of about 75 mm were dipped from a tank containing the stock of goldfish, measured, and put in duplicate experimental tanks (A and B). Usually two pike of 343–383 mm, measured before or after an experiment, were added to each tank ½ or more hours later. Some pike were used in successive experiments. Goldfish were anesthetized with tertiary amyl alcohol for handling in Experiment 2 only. Goldfish were fed dry commercial fish food during the experi-

[1]U.S. Bureau of Sport Fisheries and Wildlife, Wisconsin Department of Natural Resources, and University of Wisconsin, Stevens Point, cooperating.

[2]Some of the green sunfish, perhaps 50%, were not true species, but were hybrids between green sunfish and, probably, bluegill. They resembled green sunfish in appearance more than any other species.

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ments. Experiments ended in 9–19 days when fish were removed, identified, and measured.

Satiation of predator — Three experiments were conducted from January 25 to May 1, 1972, in circular plastic pools at the University of Wisconsin in Stevens Point, Wisconsin. The pools were 2.4 or 3.0 m in diameter and contained about 1700 or 3000 liters of well water. Each was provided with an electric heater, an air stone, and one or two shelters, and each received a slow flow of water that ranged in temperature from 10 to 18 C and averaged 15 C. Shelters were made of strips of black polyethylene, which were anchored and extended vertically through the water to resemble an aquatic plant. Shelters varied from about 50 to 100 cm in diameter at the surface where the free end of the strips floated. Sheets of plywood (1.2 × 2.4 m) were put on the pools to provide additional cover but were not placed in such a way as to exclude natural light.

Prey fishes were obtained from the Lake Mills (Wis.) National Fish Hatchery of the U.S. Bureau of Sport Fisheries and Wildlife and northern pike from Rush Lake, Wis.

Four species of prey were presented to the pike immediately after satiation of the predator, after 15–17 days food deprivation, and after 29 days deprivation. In the satiated treatment each pike was held individually in a pool for 17 days and fed ab libitum. An attempt was made to maintain 4 or 6 goldfish with each pike; as goldfish were eaten, they were replaced. Observations began following a period in which the pike left goldfish uneaten after they were known to have recently fed.

Ten each of carp, fathead minnow, green sunfish, and bluegill of about 48 mm were anesthetized with tertiary amyl alcohol, measured, and put in duplicate pools (A and B) 4–12 hr before a pike of about 400 mm was added to each pool. Additional prey, treated in the same manner, were put in a cage in another pool without a pike to determine the extent of nonpredatory mortality. Experiments ended in 2–7 days when fish were removed, identified, and measured.

Results and discussion — Appearance of prey — Vulnerability to northern pike predation was the same for fantail and regular-tail goldfish. The pike ate 29 of 90 of each kind of goldfish (Table 1).

Color did not influence vulnerability of the prey (Table 2). Of 60 each of gold and natural color goldfish offered in Experiment 2, the pike ate 19 gold and 22 natural color; the difference is not significant ($\chi^2 = 0.645$ with 1 df). When three colors of prey were used in Experiment 3, the northern pike did eat more natural color than gold or white goldfish, but differences among the three were not significant ($\chi^2 = 3.501$ with 2 df).

The northern pike seemed to have a tendency to eat the smaller goldfish. Mean lengths were larger at

TABLE 1. Number of fantail and regular-tail goldfish (*Carassius auratus*) eaten by northern pike (*Exos lucius*), 348–377 mm long, in Experiment 1. Fifteen of each kind of goldfish, all of natural color, were put in each tank. A and B are replicate tanks.

Run	Tank	Duration (*days*)	No. eaten	
			Fantail	Regular-tail
1	A	17	6	6
	B	13	5	1
2	A	9	3	3
	B	9	6	6
3	A	19	5	4
	B	19	4	9
Total			29	29

TABLE 2. Number of goldfish eaten by northern pike, 343–383 mm long, in Experiments 2 and 3. Fifteen each of gold and natural color goldfish, all fantail, were used in Experiment 2; and 10 each of gold, natural, and white goldfish, all regular-tail, were used in Experiment 3. A and B are replicate tanks.

Experiment	Run	Tank	Duration (*days*)	No. eaten		
				Gold	Natural	White
2	1	A	8	3	4	
		B	8	4	4	
	2	A	11	2	5	
		B	11	10	9	
Total				19	22	
3	1	A	10	8	8	5
		B	10	2	7	2
Total				10	15	7

the end than the beginning of most experiments (Table 3), and the increase in length was significant (t = −7.974 with 11 df in Experiment 1 and t = −2.785 with 13 df in Experiments 2 and 3). A comparison of length-frequency distributions for goldfish at the beginning and end of the experiments indicated that the prey may have grown in Experiment 1 but not in Experiments 2 and 3. Perhaps there was both growth of goldfish and selection of smaller prey by the predator. The latter would explain the higher vulnerability of natural color goldfish in Experiment 3. Those fish were significantly smaller than the gold and white goldfish (t = −4.196 with 58 df).

TABLE 3. Mean length (*mm*) and standard deviation (in parentheses) of fantail (F), regular-tail (R), gold (G), white (W), and natural (N) color goldfish at the beginning and end of Experiments 1–3. Data for replicate tanks are combined.

Experiment	Run	Beginning	End
1	1	F71(4)	73(4)
		R71(5)	75(5)
	2	F72(7)	74(6)
		R71(6)	74(6)
	3	F72(7)	75(7)
		R72(7)	74(8)
2	1	G71(4)	73(5)
		N70(5)	71(7)
	2	G73(4)	77(3)
		N70(5)	73(5)
3	1	G77(9)	78(9)
		N71(6)	72(7)
		W81(6)	80(7)

Beyond the "leaping distance" of pike, or about 10 cm depending on the size of the animal, northern pike feed by sight (Hoogland et al. 1956). There is little doubt that pike perceive by sight differences among prey e.g. Hoogland et al. (1956) found that in response to visual stimuli northern pike learned to avoid sticklebacks before touching them. Such perception could include differences in behavior or manner of movements and in physical appearance — form, brightness, pattern of dark and light, and color. Results of the first three experiments indicate that differences in form (of the tail), brightness (white vs. natural color), and color are not important to northern pike.

Behavior or manner of movement of prey probably is important to pike. Hoogland et al. (1956) felt

TABLE 4. Number of prey eaten by satiated and starved northern pike, 362–415 mm long, when 10 fish of each species of prey and one pike were put in each pool. A and B are replicate pools.

Experiment	Duration (*days*)	Days of food deprivation	Carp (*Cyprinus carpio*)	Fathead minnow (*Pimephales promelas*)	Green sunfish (*Lepomis cyanellus*)	Bluegill (*L. macrochirus*)
4 A	4	0	3	5	0	0
B	7	0	8	8	0	1
5 A	2	15	7	8	0	0
B	2	17	6	1	0	0
6 A	4	29	0	8	0	0
B	4	29	7	8	0	0

TABLE 5. Mean length (*mm*) and standard deviation (in parentheses) of prey at the beginning and end of Experiments 4–6. A and B are replicate pools. A dash (–) indicates that mean length was the same at the end as at the start of an experiment since no fish were eaten.

	Carp		Fathead minnow		Green sunfish		Bluegill	
Experiment	Start	End	Start	End	Start	End	Start	End
4 A	45 (5)	42 (4)	50 (4)	47 (2)	50 (3)	–	49 (3)	–
B	52 (11)	51 (4)	56 (8)	56 (6)	49 (3)	–	49 (4)	48 (4)
5 A	48 (4)	45 (2)	47 (2)	47 (2)	47 (5)	–	46 (2)	–
B	48 (4)	47	46 (2)	46 (2)	48 (3)	–	47 (2)	–
6 A	56 (14)	–	53 (8)	49 (10)	50 (4)	–	52 (5)	–
B	57 (16)	50 (9)	52 (7)	55 (13)	51 (4)	–	52 (3)	–

that the way a stickleback moves was the main characteristic of the visual stimulus that pike became conditioned to avoid. Physical appearance rather than behavior was studied in these experiments, but if the behavior of goldfish is the same regardless of the form of their tail or their color, then the results of these experiments are consistent with the hypothesis that behavior is more important than physical appearance in influencing the predator.

Satiation of predator — Food deprivation did not affect food selection of northern pike (Table 4). Carp and fathead minnow were much more vulnerable to the satiated pike than green sunfish and bluegill, as had been found by Mauck and Coble (1971). Relative vulnerability of the prey was the same after about 2 and 4 weeks of predator food deprivation.

Size of prey probably had little influence on the results. Lengths of prey were quite similar within experiments, and there were small and inconsistent changes in length from the beginning to the end of the experiments (Table 5).

Mauck and Coble (1971) found that northern pike selected certain species of fish, usually the nongame fishes, over others. Their pike probably were satiated. Although they presented evidence that pike feeding in nature may be like that observed in their experiments, the question remained whether or not less satiated northern pike in nature would be as selective in their feeding. Results of Experiments 4–6 indicate that the degree of satiation of northern pike does not influence species selection of prey by the predator.

Degree of satiation may influence size selection of a predator; however, Nakamura (1962) found that after skipjack tuna (*Euthynnus pelamis*) consumed a few whole shrimp or squid, they refused further offerings of the same size but accepted them when cut into smaller pieces.

Acknowledgments — Students G. Vaughan, L. McLain, N. Emerick, C. Prentiss, and D. Smart helped identify, count, and measure fish. I was associated with the Missouri Cooperative Fishery Unit when the first three experiments were carried out. Northern pike used in the second set of experiments were captured by personnel of the Wisconsin Department of Natural Resources. Dr J. J. Magnuson, University of Wisconsin, Madison, read the manuscript.

HOOGLAND, R., D. MORRIS, AND N. TINBERGEN. 1956. The spines of sticklebacks (*Gasterosteus* and *Pygosteus*) as means of defense against predators (*Perca* and *Esox*). Behaviour 10: 206–236.

MAUCK, W. L. AND D. W. COBLE. 1971. Vulnerability of some fishes to northern pike (*Esox lucius*) predation. J. Fish. Res. Board. Can. 28: 957–969.

NAKAMURA, E. L. 1962. Observations on the behavior of skipjack tuna, *Euthynnus pelamis*, in captivity. Copeia 1962: 499–505.

Vers une analyse objective de la thermocline saisonnière

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BOUDREAULT, F.-R., ET J.-P. LAPRISE. 1973. Vers une analyse objective de la thermocline saisonnière. J. Fish. Res. Board Can. 30: 320–322.

Nous proposons une définition de la thermocline saisonnière, à partir de la généralisation suggérée par Riffenburgh (1970). Celle-ci permet une analyse objective de la thermocline.

BOUDREAULT, F.-R., ET J.-P. LAPRISE. 1973. Vers une analyse objective de la thermocline saisonnière. J. Fish. Res. Board Can. 30: 320–322.

We propose a definition of the seasonal thermocline, based on a generalization suggested by Riffenburgh (1970). This allows objective analysis of the thermocline.

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NOTRE propos vise à définir la thermocline saisonnière d'une manière qui permette l'analyse objective et quantitative.

Pour ce faire, la méthode de Boston (1966) présente, en particulier, deux inconvénients:

1, chaque profil thermique, ou partie de celui-ci, est comparé à un profil "idéal" dérivé d'une courbe gaussienne, ce qui présume ou du mécanisme de formation, ou de la loi de distribution de la température;

2, un profil digitalisé au 0,1 C produit un grand nombre de points d'inflexion (dérivée seconde nulle), d'où la nécessité de sectionner le profil en autant de parties dont on devra choisir laquelle représentera la thermocline saisonnière.

Nous croyons plus prometteur l'approche statistique de Riffenburgh (1970), lequel suggère que "... in the absence of precise boundaries, we can often speak of probable location of boundaries" (Riffenburgh 1970 p. 303). Dans cette optique, la thermocline se définit ainsi: une couche approximativement horizontale dans laquelle le gradient vertical de température est significativement plus grand que le gradient moyen du profil.

L'expression "significativement plus grand que" peut correspondre à des niveaux différents selon

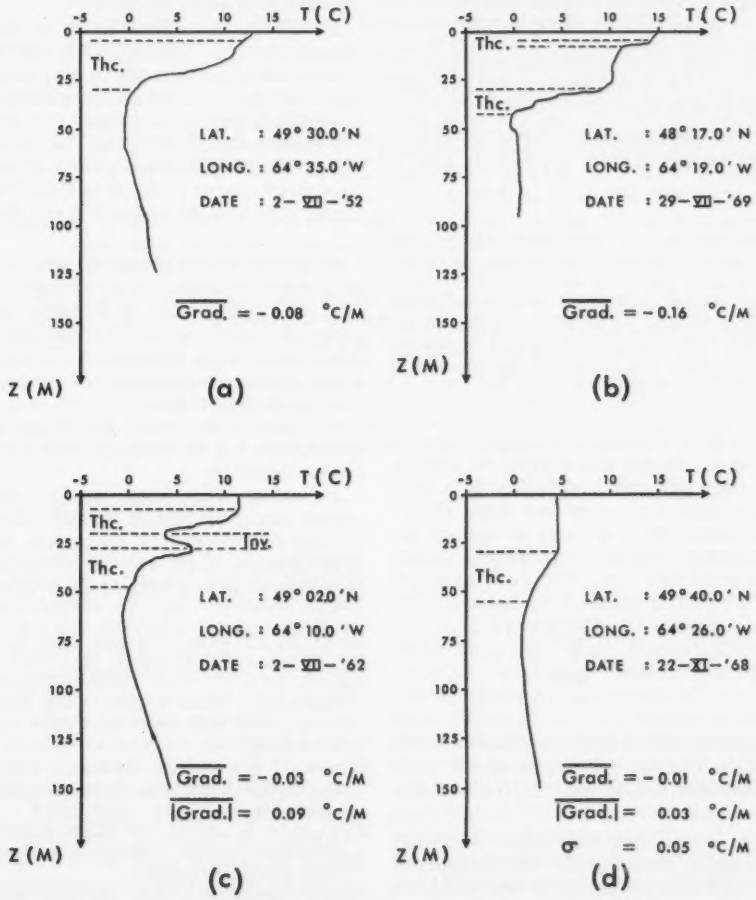

FIG. 1. Illustration de cas-types de la thermocline saisonnière dans le golfe du Saint-Laurent: (a) Thermocline (Thc.) estivale simple; (b) double; (c) avec Inversion (Inv.) et (d) automnale simple.

que l'on désirera parler de thermoclines transitoires, saisonnières ou permanentes. De même, le "gradient moyen" peut être obtenu à partir des valeurs algébriques ou absolues des gradients, ou de toute autre façon de mesurer la 'moyenne' d'une distribution. Ainsi, en associant la façon suivant laquelle la moyenne est déterminée avec le niveau de différence par rapport à cette dernière, divers 'genres' de thermoclines pourront être analysés.

Application à la thermocline saisonnière du golfe du Saint-Laurent — Les bathythermogrammes (BT) provenant du golfe du Saint-Laurent sont digitalisés par le Centre canadien des Données océanographiques, de la façon suivante: en partant de la surface, et pour chaque changement de température de ± 0,1 C, on note, de façon consécutive, les valeurs correspondantes de la profondeur (Jones 1970). Ce mode entraîne, dans les zones de forts gradients, des valeurs consécutives identiques de la profondeur, d'où le gradient $(\Delta T/\Delta Z) \rightarrow \infty$.

Nous convertissons donc ces profils en une séquence de températures (T_n) correspondant aux profondeurs successives $n \cdot \Delta Z$ ($n = 1,2,\ldots\ldots MT$; ΔZ = intervalle minimum de profondeur; $MT \cdot \Delta Z$ = profondeur maximum).

L'intervalle minimum de température est déjà fixé à 0,1 C, et comme nous ne désirons point analyser la microstructure, nous prendrons un intervalle minimum de profondeur de 2,5 m (Cooper et Stommel 1968).

Ceci implique une valeur minimum du gradient thermique ($|\Delta T/\Delta Z|$ Min = 0,04 C/m) de sorte que toute valeur du gradient sera un multiple entier de cette valeur-limite.

Résultats — Afin d'illustrer les possibilités de cette approche, un premier essai a été fait en utilisant la moyenne algébrique dans la définition de la thermocline saisonnière. Celle-ci devient donc: l'intervalle de profondeur où le gradient est égal ou supérieur au gradient algébrique moyen du profil, soit

$$\overline{\text{Grad}} \geqslant \frac{1}{MT-1} \sum_{n=1}^{MT-1} G_n$$

Nous constatons que ce simple choix donne déjà des indications fort intéressantes pour une thermocline saisonnière unique (Fig. 1a) ou multiple (Fig. 1b).

Cependant, la présence de fortes inversions (gradients positifs) entraîne une telle diminution du gradient algébrique moyen, que ce dernier devient inférieur à la valeur-limite pour laquelle notre définition de la thermocline saisonnière est valable.

L'emploi de la moyenne des gradients absolus

$$\overline{|\text{Grad}|} \geqslant \frac{1}{MT-1} \sum_{n=1}^{MT-1} |G_n|$$

contourne cette difficulté (Fig. 1c).

Limitations — 1° Si nous limitons notre analyse à la partie saisonnière du profil, soit approximativement 150 m (Banks 1966), la présente méthode apparaît satisfaisante. Si nous allons plus profondément, disons jusqu'à 250 m, le gradient algébrique moyen et/ou le gradient absolu moyen devient inférieur à la valeur-limite.

2° Un examen préliminaire de 343 BT provenant du sud-ouest du Golfe (48°–49°N; 64°–65°W) indique que notre méthode, telle quelle, ne couvrira pas, dans une étude statistique, la période qui va de novembre à avril inclusivement.

L'examen de 290 BT pour l'est du Golfe (47°–48°N; 59°–60°W) indique aussi que notre analyse ne pourrait couvrir d'une façon statistiquement adéquate, la période de décembre à mai inclusivement.

Remarques — Ces limitations peuvent disparaître en utilisant un autre type de moyenne, soit l'écart-type. Si, comme nous le croyons, l'introduction arbitraire de cette moyenne conduit pour la période juin–octobre à des résultats statistiques semblables à ceux obtenus par les deux moyennes déjà proposées, nous pourrons, en plus,

1° analyser les profils thermiques jusqu'à des profondeurs où la structure demeure stable, à l'année longue, et

2° inclure dans notre analyse statistique la période automnale tardive, période pendant laquelle il existe une thermocline saisonnière, (ex. Fig. 1d) et du printemps (avril–mai) qui, toutes deux, échappent présentement à notre analyse saisonnière.

BANKS, R. E. 1966. The cold layer in the Gulf of St-Lawrence. J. Geophys. Res. 71(6): 1603–1610.

BOSTON, N. E. J. 1966. Objective definition of the thermocline. Texas A. & M. Univ., College Station, Texas. Tech. Rep. 286-D: ix + 38 p. (Unpublished MS Report)

COOPER J. W., AND H. STOMMEL. 1968. Regularly spaced steps in the main thermocline near Bermuda. J. Geophys. Res. 73(18): 5849–5854.

JONES, H. A. C. 1970. The CODC Bathythermograph digitization system. Operator's manual. (Unpublished internal document)

RIFFENBURGH, R. H. 1970. Probable depths of interfaces between temperature layers, with a generalization. Deep-Sea Res. Oceanogr. Abstr. 17: 303–316.

Survival and Gill Condition of Bluegill (*Lepomis macrochirus*) and Fathead Minnows (*Pimephales promelas*) Exposed to Sodium Nitrilotriacetate (NTA) for 28 Days

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MACEK, K. J., AND R. N. STURM. 1973. Survival and gill condition of bluegill (*Lepomis macrochirus*) and fathead minnows (*Pimephales promelas*) exposed to sodium nitrilotriacetate (NTA) for 28 days. J. Fish. Res. Board Can. 30: 323–325.

Acute dynamic bioassays with fathead minnows and rainbow trout indicated that the 96-hr median tolerance limits of sodium nitrilotriacetate (NTA) exceed 1000 times the mean environmental levels that might be anticipated from detergent use. A 28-day dynamic bioassay with bluegill and fathead minnows indicated a lack of cumulative toxicity associated with levels of NTA up to 1000 times expected environmental concentrations in water. Fishes exposed to 96 mg/liter NTA for 28 days exhibited no NTA-induced gill pathology.

MACEK, K. J., AND R. N. STURM. 1973. Survival and gill condition of bluegill (*Lepomis macrochirus*) and fathead minnows (*Pimephales promelas*) exposed to sodium nitrilotriacetate (NTA) for 28 days. J. Fish. Res. Board Can. 30: 323–325.

Des essais biologiques effectués sur des crapets à oreilles bleues, des ménés à grosse tête et des truites arc-en-ciel démontrent que la médiane de la limite de tolérance, après 96 h, du nitrilotriacétate de sodium (NTA) dépasse de 1000 fois les niveaux moyens qu'on peut s'attendre de trouver dans le milieu ambiant par suite de l'emploi de détersifs. Un essai de 28 jours avec le crapet à oreilles bleues et le ménés à grosse tête révèle une absence de toxicité cumulative associée à des concentrations de NTA allant jusqu'à 1000 fois celles qu'on peut rencontrer dans le milieu aquatique. Les branchies de poissons exposés à une concentration de NTA de 96 mg/litre durant 28 jours ne montrent aucun symptôme pathologique attribuable au NTA.

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TRISODIUM nitrilotriacetate (NTA) is considered an effective substitute for sodium tripolyphosphate in synthetic detergents. Average anticipated percent composition is about 10% of a detergent product. Since typical dilution from detergent product to municipal sewage treatment influent is 1:20,000, it is estimated that a concentration of 5 mg/liter NTA will be entering sewage treatment facilities. The above dilution calculations are based on the total annual detergent production and household water usage for the United States (detergent products 1.81×10^9 kg/year; domestic water sewage 4.35×10^{13} liters/year). It has been demonstrated that more than 90% of NTA is degraded during activated sludge treatment, both in the laboratory (Swisher et al. 1967; Thompson and Duthie 1968), and in operating wastewater treatment plants (Shumate et al. 1970); and that the degradation of NTA is essentially complete to the end products of CO_2 and inorganic N (Thompson and Duthie 1968). Thus, the average expected levels in sewage treatment plant effluent are approximately 0.5 mg/liter. Assuming a mean dilution of sewage treatment plant effluent of 1:10, the average expected level of NTA in receiving water immediately downstream from the treatment plant is 0.05 mg/liter.

J. E. Weaver[1] (unpublished data) has shown the acute toxicity (96-hr TL_{50}) of NTA in water of 60 mg/liter $CaCO_3$ hardness to bluegill (*Lepomis macrochirus*), snails (*Physa heterostropha*), and the diatom (*Navicula seminulum*) to be 252 mg/liter, 373 mg/

[1]J. E. Weaver, Procter & Gamble Co., Cincinnati, Ohio.

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liter, and 185 mg/liter, respectively; approximately three orders of magnitude higher than the average expected levels in receiving waters. We have extended these acute toxicity studies to rainbow trout (*Salmo gairdneri*) and fathead minnows (*Pimephales promelas*) in soft (35 mg/liter as $CaCO_3$) water. Additionally, we have evaluated the toxicity of NTA to bluegill and fathead minnows under conditions of 28 days' continuous exposure to measured concentrations of NTA ranging from a mean (SE) of 3.4 (0.2) to 172.8 (3.7) mg/liter.

Tests were conducted in a continuous-flow proportional dilution apparatus (Mount and Brungs 1967) which provides for the automatic intermittent introduction of the test material and diluent water into the test chambers. The system delivered eight chemical concentrations (Table 1) and provided diluent water to a control unit. Flow rate to each 30-liter test chamber was 6 liters/hr. The desired chemical concentrations were initially established by adding sufficient amounts of a chemical stock solution (Na salt of nitrilotriacetate dissolved in water) to each test chamber. Thereafter, the proportional dilution apparatus was used to maintain the desired chemical concentration.

During the 28-day study, water samples for NTA analysis were taken at 96-hr intervals from three tanks containing nominal concentrations of 200 mg/liter, 72 mg/liter; and 5.6 mg/liter, of NTA. These samples were preserved with 1% formalin and shipped under refrigeration to the Procter and Gamble Company, Cincinnati, Ohio for analysis. Samples were not taken from tanks containing the five additional levels of NTA. NTA was analyzed using the Zinc–Zincon method for sequestrant in waste and sewage, as described by Thompson and Duthie (1968). All NTA values are reported as mg/liter anhydrous trisodium NTA (MW=257).

Twenty bluegills (mean wt. 1.5 g) and 20 minnows (mean wt. 0.8 g) were tested at each concentration. Test fish were observed in the laboratory for a minimum of 21 days prior to testing. During that period, mortality in the test population was less than 2% and the fish were judged to be in excellent physical condition. The tests were conducted at 19 C ($\pm$0.5) except for trout which were tested at 13 C ($\pm$0.5). The diluent water consisted of deionized water of at least 1 million ohms resistivity which was reconstituted by adding 3 mg potassium chloride, 30 mg calcium sulfate, 30 mg magnesium sulfate, and 48 mg sodium bicarbonate per liter. The total hardness of the test diluent was 35 ppm as $CaCO_3$, the *p*H was 7.1, and mean dissolved oxygen was 5.2 ppm (range 4.3–5.7 ppm). During the 28 day-study, the fish were fed a dry pelleted fish ration ad libitum each day.

TABLE 1. Cumulative mortalities of bluegill (*Lepomis macrochirus*) and fathead minnows (*Pimephales promelas*) during 28 days' exposure to various concentrations of NTA (20 fish per concentration).

Concentration (*mg/liter*)		% Mortality	
Nominal	Measured or Recalculated[a]	Fathead Minnow	Bluegill
Control		10	10
200	172.8(144.0–196.0)[b]	100	55
120	96.0	0	10
72	56.4(46.9–65.6)	0	0
43.2	29.4	0	5
25.9	17.1	0	0
15.5	10.0	0	5
9.3	6.0	0	15
5.6	3.4(2.3–5.8)	5	15

[a]Recalculated on the basis of least squares regression analysis of all data from water monitoring.
[b]Mean measured concentration (range).

Dynamic bioassays in soft water (35 mg/liter as $CaCO_3$) indicated the 96-hr median tolerance limit (95% confidence interval) for NTA was 98 (72–133) mg/liter for rainbow trout, and 127 (93–170) mg/liter for fathead minnow.

The results of the 28-day dynamic bioassay were consistent with the results of the acute bioassays. During the first 48 hr exposure to 172.8 mg/liter NTA, all of the minnows, and 40% of the bluegill succumbed; mortality among bluegill (Table 1) did not increase appreciably after that time. During the first 9 days of the test no mortality was observed with either species at any of the other exposure levels (3.7–96.0 mg/liter) tested and no appreciable mortality occurred at any of these levels during the 28-day exposure to NTA. In both species cumulative mortality due to continuous exposure to NTA was conspicuously absent, and the fishes appeared healthy and normal.

In order to evaluate possible NTA-induced damage to gills tructure, fish were killed after 28-days exposure and fixed in Bouin's fixative. After dehydration, embedding in paraffin, and sectioning, a minimum of five sections from each gill were stained using Heidenhain's Azan technique. Microscopic examination of mounted gill sections indicated no changes in gill histology occurred due to 28 days of continuous exposure to concentrations of NTA up to 96 mg/liter. In addition, fish exposed continuously to NTA for 28 days were transferred to clean water for 7 days and gill sections prepared for histological examination. No NTA-induced gill pathology was evident.

It appears that 28 days' continuous exposure to levels of NTA 1000 times higher than expected environmental levels had no significant effect on gill structure or survival of bluegill and minnows.

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MOUNT, D. I., AND W. A. BRUNGS. 1967. A simplified dosing apparatus. Water Res. 1: 21–29.

SHUMATE, K. S., J. E. THOMPSON, J. D. BROOKHART, AND C. L. DEAN. 1970. NTA removal by activated sludge — a field study. J. Water Pollut. Contr. Fed. 42: 631–640.

SWISHER, R. D., M. M. CRUTCHFIELD, AND D. W. CALDWELL. 1967. Biodegradation of nitrilotriacetate (N TA) in activated sludge. Environ. Sci. Technol. 1: 820–827.

THOMPSON, J. E., AND J. R. DUTHIE. 1968. Biodegradability and treatability of NTA. J. Water Pollut. Contr. Fed. 40: 306–318.

Speed, Stamina, and Muscles in Fishes

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GATZ, A. J., JR. 1973. Speed, stamina, and muscles in fishes. J. Fish. Res. Board Can. 30: 325–328.

A new interpretation is offered of some of the classic experiments on swimming in fishes done by Bainbridge. It is suggested that certain aspects of his results can be explained best by recognizing the discrete functions of the red and white muscle fibers in fishes. Persons investigating swimming performance of fishes are cautioned to bear in mind these distinctions in future work.

GATZ, A. J., JR. 1973. Speed, stamina, and muscles in fishes. J. Fish. Res. Board Can. 30: 325–328.

L'auteur propose une nouvelle interprétation de certaines expériences classiques faites par Bainbridge sur la nage des poissons. Il prétend que certains aspects particuliers des résultats de Bainbridge s'expliquent mieux si on reconnaît des fonctions distinctes aux fibres musculaires rouges et blanches chez les poissons. Les chercheurs qui étudient la performance natatoire des poissons devraient tenir compte de ces distinctions au cours de leurs travaux futurs.

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BODDEKE et al. (1959) demonstrated that the composition of the myotome in terms of amounts and locations of red and white fibers is related to behavior and habitat preferences in a number of species of fishes. From their results one might expect also that muscular type would be crucial to the swimming performance of a fish in any given experimental test. However, most of the work on muscle fiber function in fishes was published after Bainbridge's classic contributions to fish locomotion; thus Bainbridge (1958, 1960, 1962) did not have the benefit of these other workers' conclusions in interpreting his own results. The purpose of this note is to indicate how a consideration of the roles of red and white muscle, a consideration which Bainbridge did not make, might help to explain some of his results.

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This synthesis is felt to be needed because Bainbridge's work seems to have become a standard in the literature of fish locomotion. First, I shall review some of the evidence concerning how the two fiber types function, then I shall proceed to indicate where this knowledge might aid in understanding Bainbridge's results.

It is well established (Bokdawala 1967; Bokdawala and George 1967a, b; Bilinski 1969; Dubowitz and Pearse 1960; and Bone 1966 and references cited therein) that histological and histochemical differences exist between various muscle fibers in fishes. However, such work provides only circumstantial evidence that red and white fibers function differently. Direct support also exists for the functional duality first hypothesized for fishes nearly 100 years ago by Arloing and Lavocat (1875), that red fibers are used in slow sustained swimming while the more

abundant white fibers are employed only in sudden bursts of activity. Pennycuick in 1962 (Bone 1964) and Rayner and Keenan (1967) measured muscle action potentials in swimming fishes. In both studies, the investigators found that the red fibers were active and the white fibers were not in slow swimming. The white fibers became active only when fishes made sudden movements or were stimulated tactilely. Alexander (1969) discussed the differences in red and white fiber arrangement which are associated with their contractile differences.

Additional evidence for differential function of the red and white fibers has come from investigation of the concentrations of energy sources in the muscles before and after exercise. The idea that fat is used by fishes during spawning migrations and that the red muscle is the site of storage for this fat is not new (Paton 1898; Greene 1912, 1913, 1921; Wilson 1939), but has been studied by modern techniques (Chang and Idler 1960). Other recent investigators have considered the white muscle fibers. Wittenberg and Diacinc (1965) electrically stimulated muscle directly to produce different levels of effort. They monitored changes in concentration of glycogen and its metabolites and suggested that the red muscle was the "burning place" of catabolites produced in the white muscle fibers during strenuous exercise. Black et al. (1961) found rapid depletion of white muscle glycogen to ½ resting level after strenuous exercise, but no change in muscle glycogen in yearling salmon that had been sustained for twice as long at a moderate level of activity, comparable to a migration run. Connor et al. (1964) found muscle glycogen depletion in salmon and trout ascending a 1:8 fishway, but not in those ascending a 1:16 fishway. All these studies, then, indicate that moderate or sustained activity draws on the red muscles via fat metabolism, while more strenuous demands or rapid bursts cause glycogen utilization by the white fibers.

Further evidence of muscle fiber function has come from studies of respiration. Smit et al. (1971) investigated the relationship between efficiency of swimming (= power/net O_2 uptake) and swimming speed in goldfish. They calculated that efficiencies greater than 20%, corresponding to speeds greater than 3.4 body lengths per second (L sec^{-1}), are indicative of at least some energy being derived anaerobically. That is, assuming red fibers function aerobically, speeds of 3.4 L sec^{-1} are the maximum sustainable by red fibers alone so that at higher speeds both red and white fibers must be functioning together. Webb (1971) did similar experiments using rainbow trout. He found that maximum aerobic efficiency was about 16% and that at 2 L sec^{-1} only about 4% of the energy being used came from anaerobic sources. At speeds below 80% of this critical speed (< 1.6 L sec^{-1}) the anaerobic energy contribution was negligible, that is, the white muscles seemed not to be functioning. Kutty (1968) found that the respiratory quotient (R.Q. = vol. of CO_2 produced/vol. of O_2 consumed) did not exceed one in either goldfish or rainbow trout swimming in $\geq 50\%$ air-saturated water at speeds up to 3.6 L sec^{-1} to 5.1 L sec^{-1}, respectively. These speeds and R.Q.'s were maintained for 3–12 hr in the different test groups. Kutty concluded that the energy being used for this sustained swimming came from aerobic metabolism. In that Kutty did not determine any R.Q.'s at higher swimming speeds, it is not known at what speeds R.Q.'s greater than unity indicative of anaerobic respiration might be found. Still, all three studies do provide some additional evidence of the aerobic nature of sustained swimming which is consistent with other knowledge of red fiber function.

Bainbridge (1958) related speed of swimming to size of fish and frequency and amplitude of tail beat in three species: dace, *Leuciscus leuciscus;* rainbow trout, *Salmo gairdneri* (= *S. irideus*); and goldfish, *Carassius auratus*. In his discussion, Bainbridge indicated that the formula $V = \frac{1}{4}\{L(3f-4)\}$ described the swimming velocities for all three species at $f >$ ca. 5, where V = speed in cm sec^{-1}, L = body length in centimeters, and f = tail frequency in beats sec^{-1}. Thus, at speeds of 3 L sec^{-1} and higher, all three species performed equivalently. Natural cruising speed for most species seems to be 2–3 L sec^{-1} (Bainbridge 1960), so it would seem that the formula applies only to speeds which should be considered to be burst speeds and which are, therefore, most likely attained by use of the white muscles. As Boddeke et al. (1959) observed, white fibers always form the major part of the musculature so that "in principle all fishes are built for the sprint." The similarity of performance Bainbridge observed at higher speeds might thus be anticipated due to the general similarity of musculature for higher speeds.

Correspondingly, the differences Bainbridge found between species at lower frequencies up to 3–4 beats sec^{-1} or speeds up to about 2 L sec^{-1}, i.e. cruising speeds, might result from the difference in amount of red muscle present in the species used. To investigate this possibility, I determined the percentage of red muscle fibers in the myotome of these fishes. I made freezing microtome sections through the middle of the caudal peduncle of formalin-fixed fishes, stained for fat to identify the red fibers, and determined the area of the axial musculature comprised in each section by each fiber type. I found *Salmo gairdneri* to have 12.5% ($\pm$ 0.7 = $2s_{\bar{x}}$) and

Carassius auratus to have 5.8% ($\pm 1.3 = 2s_{\bar{x}}$). I did not have *Leuciscus leuciscus* available for study, but based on its external anatomy, habits, and habitat preferences (Wheeler 1969), I would estimate it to be most similar in red muscle content to *Semotilus atromaculatus* and *Clinostomus funduloides* of the fishes I have studied. These fishes have 7.7 and 9.2% red muscle, respectively, so it seems likely that dace would have about 8.5% red fibers in the peduncle region. Bainbridge's (1958) results show that trout, "stayers" (Boddeke et al. 1959), are capable of maintaining the same amplitude over all frequencies (Bainbridge 1958, fig. 10), whereas in goldfish amplitude drops as frequency drops (Bainbridge 1958, fig. 14), and dace (Bainbridge 1958, fig. 6), "crawlers" (Boddeke et al. 1959), are intermediate. The significance here is that any one dace or trout exhibits less variation in tail beat amplitude at low speeds (frequencies), and maintains a more constant distance per beat than a goldfish (Bainbridge 1958, fig. 5, 9, and 13). That is, the fishes with more red muscle perform better at low speeds than do goldfish, which have the least red muscle.

Bainbridge (1960) reported speed–duration data for fishes of the same three species. For the size ranges used and 1-sec duration, he found dace able to go to 11–13 L sec^{-1} and trout and goldfish both able to go 9–12 L sec^{-1}. However, 20 sec after starting, speeds dropped to 4–4.5 L sec^{-1} for dace, 2.5–3.0 L sec^{-1} for trout, and 3.8–6.5 L sec^{-1} for goldfish. In discussing these interspecific differences, Bainbridge states, "The differences appear to be real and one may be forced to invoke some hitherto undetermined hydrodynamical or physiological factor to account for them." I suggest that here, too, differences in muscle fiber composition among the species might provide a partial explanation. Little difference in top speed for the shortest bursts exists. Results for all species are in the magnitude of 10 L sec^{-1}. However, after the first few seconds, the lack of white muscle mass becomes evident in the trout, the stayers. The trout are the first to lose their ability to sprint, so that after only 15 sec they have slowed to only slightly faster than the 2 L sec^{-1} they can maintain "... for periods up to 5 min and probably a matter of hours" (Bainbridge 1962). Goldfish and dace, with higher percentages of white muscle, are able to sprint faster longer, but their speeds in body lengths sec^{-1} are dropping more rapidly than those of the trout over the 15–20-sec time interval (Bainbridge 1960, fig. 10). Unfortunately, Bainbridge did not test these species for times any longer than 20 sec, so one does not really know from his work how speeds after 5 min or more would compare with the trout. As previously mentioned, however, Kutty (1968) found trout able to sustain a higher speed (5.1 sec^{-1}) than goldfish (3.6 L sec^{-1}) for a period of hours. This result is consistent with the interpretation that an increase in amount of red muscle should permit an increase in sustained swimming ability. I should also point out that many of the physiological factors Bainbridge suggested should influence the time/speed relationship are factors which vary between red and white muscle fibers.

Bainbridge (1962) investigated the effect of training on swimming speed in trout by repeating his earlier experiments, but using trout which had been swimming continuously at 1.0–1.5 L sec^{-1} for a year. His results showed no differences in speeds achieved in the 1–20 sec testing time. He also tested eight fish for longer durations (32–104 min), but had no data for untrained fish with which to compare these results. Although Bainbridge made many good points about the apparent physiology of the situation which produced the observed results, he was unable to offer a convincing explanation for them. I feel that such an explanation can be provided if one considers the roles of the red and white fibers in both the training and the testing processes. The low cruising speed required during training assuredly could be met by the red fibers alone in a stayer like the trout. Thus, the white muscles of the trained trout were not exercised any differently than in the unexercised trout maintained in smaller tanks which made brief bursts from time to time. The test situation, as previously emphasized, is one which necessitates white muscle fiber function; hence, no difference in performance should be expected.

Additional evidence for this interpretation is provided by work done by other investigators, using other salmonids which had been conditioned similarly. Brett et al. (1958) compared cruising speeds (minimum test period of 1 hr) in exercised and unexercised coho salmon, *Oncorhynchus kisutch*, and found the exercised fish swam significantly faster. Here then, exercise does lead to improved performance when the exercised muscles are tested. Hochachka (1961) found that trained rainbow trout, *Salmo gairdneri*, took longer to fatigue, developed a greater O_2 debt, used more glycogen, had more blood hemoglobin and larger hearts, and had a shorter recovery time from fatigue than did untrained fish. These results are consistent with the idea of a greater functional capacity in the red muscles of the trained than the untrained fish (Wittenberg and Diacinc 1965).

The interpretation of Bainbridge's results suggested above is, of course, just that — an interpretation. Although all of his results cited correspond well with what is known about red and white muscle fiber function and the composition of the myotome

of the species involved, Bainbridge did nothing which would permit one to prove which fibers were functioning at any given time. Such information would have to be obtained by measuring muscle action potentials, changes in metabolite levels, or respiratory quotients in conjunction with the locomotory tests. The importance of making these measurements should be kept in mind in future studies of swimming in fishes.

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ALEXANDER, R. M. 1969. The orientation of muscle fibres in the myomeres of fishes. J. Mar. Biol. Ass. U.K. 49: 263–290.

ARLOING, S., AND A. LAVOCAT. 1875. Recherches sur l'anatomie et la physiologie des muscles striés pâles et foncés. Mem. Acad. Sci. Belles Lettres Toulouse 7: 177–194.

BAINBRIDGE, R. 1958. The speed of swimming of fish as related to size and to the frequency and amplitude of the tail beat. J. Exp. Biol. 35: 109–133.

1960. Speed and stamina in three fish. J. Exp. Biol. 37: 129–153.

1962. Training, speed and stamina in trout. J. Exp. Biol. 39: 537–555.

BILINSKI, E. 1969. Lipid catabolism in fish muscle, p. 135–152. *In* O. W. Newhaus and J. E. Halver [ed.] Fish in research. Academic Press, Inc., New York, N.Y.

BLACK, E. C., A. C. ROBERTSON, AND R. R. PARKER. 1961. Some aspects of carbohydrate metabolism in fish, p. 89–124. *In* A. W. Martin [ed.] Comparative physiology of carbohydrate metabolism in heterothermic animals. University of Washington Press, Seattle, Wash.

BODDEKE, R., E. J. SLIJPER, AND A. VAN DER STELT. 1959. Histological characteristics of the body-musculature of fishes in connection with their mode of life. Proc. Kon. Ned. Akad. Wetensch. Ser. C Biol. Med. Sci. 62: 576–588.

BOKDAWALA, F. D. 1967. A histochemical study of fat in the red and white muscle fibers of fish skeletal muscle. J. Anim. Morphol. Physiol. 14: 231–241.

BOKDAWALA, F. D., AND J. C. GEORGE. 1967a. A histochemical study of the red and white muscles of the carp, *Cirrhina mrigala*. J. Anim. Morphol. Physiol. 14: 60–68.

1967b. A quantitative study of fat, glycogen, lipase and succinic dehydrogenase in fish muscle. J. Anim. Morphol. Physiol. 14: 223–230.

BONE, Q. 1964. Patterns of muscular innervation in the lower chordates. Int. Rev. Neurobiol. 6: 99–147.

1966. On the function of the two types of myotomal muscle fibre in elasmobranch fish. J. Mar. Biol. Ass. U.K. 46: 321–349.

BRETT, J. R., M. HOLLANDS, AND D. F. ALDERDICE. 1958. The effect of temperature on the cruising speed of young sockeye and coho salmon. J. Fish. Res. Board Can. 15: 587–605.

CHANG, V. M., AND D. R. IDLER. 1960. Biochemical studies on sockeye salmon during spawning migration. XII. Liver glycogen. Can. J. Biochem. Physiol. 38: 553–558.

CONNOR, A. R., C. H. ELLING, E. C. BLACK, G. B. COLLINS, J. R. GANLEY, AND E. TREVOR-SMITH. 1964. Changes in glycogen and lactate levels in migrating salmonid fishes ascending experimental "endless" fishways. J. Fish. Res. Board Can. 21: 255–290.

DUBOWITZ, V., AND A. G. E. PEARSE. 1960. A comparative histochemical study of oxidative enzyme and phosphorylase activity in skeletal muscle. Histochemie 2: 105–117.

GREENE, C. W. 1912. A new type of fat storing muscle in the salmon, *Oncorhynchus tschawytscha*. Amer. J. Anat. 13: 175–178.

1913. The storage of fat in the muscular tissue of the king salmon and its resorption during the fast of the spawning migration. U.S. Bur. Fish. Bull. 33: 69–138, 9 plates.

1921. Carbohydrate content of the king salmon tissues during the spawning migration. J. Biol. Chem. 48: 429–436.

HOCHACHKA, P. W. 1961. The effect of physical training on oxygen debt and glycogen reserves in trout. Can. J. Zool. 39: 767–776.

KUTTY, M. N. 1968. Respiratory quotients in goldfish and rainbow trout. J. Fish. Res. Board Can. 25: 1689–1728.

PATON, D. N. 1898. The physiology of salmon in fresh water. J. Physiol. London 22: 333–356.

RAYNER, M. D., AND M. J. KEENAN. 1967. Role of red and white muscles in the swimming of the skipjack tuna. Nature 214: 392–393.

SMIT, H., J. M. AMELINK-KOUTSTAAL, J. VIJVERBERG, AND J. C. VON VAUPEL-KLEIN. 1971. Oxygen consumption and efficiency of swimming goldfish. Comp. Biochem. Physiol. 39A: 1–28.

WEBB, P. W. 1971. The swimming energetics of trout. II. Oxygen consumption and swimming efficiency. J. Exp. Biol. 55: 521–540.

WHEELER, A. C. 1969. The fishes of the British Isles and north-west Europe. MacMillan and Co., Ltd., London. 613 p.

WILSON, D. P. 1939. Seasonal variations in the fat content of the flounder, *Pleuronectes flesus L.* J. Mar. Biol. Ass. U.K. 23: 361–379.

WITTENBERG, C., AND I. V. DIACINC. 1965. Effort metabolism of lateral muscles in carp. J. Fish. Res. Board Can. 22: 1397–1406.

A Cyprinodontid Fish, *Jordanella floridae*, as a Laboratory Animal for Rapid Chronic Bioassays

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SMITH, W. E. 1973. A cyprinodontid fish, *Jordanella floridae*, as a laboratory animal for rapid chronic bioassays. J. Fish. Res. Board Can. 30: 329–330.

The flagfish (*Jordanella floridae*) is proposed as a useful laboratory fish for chronic toxicity studies. It matures rapidly, reaching breeding age in 6–8 weeks under optimum conditions, and the fish will spawn at any time of the year. The adults are sexually dichromatic. A complete chronic test may be conducted with this species in as little as 3–4 months.

SMITH, W. E. 1973. A cyprinodontid fish, *Jordanella floridae*, as a laboratory animal for rapid chronic bioassays. J. Fish. Res. Board Can. 30: 329–330.

L'auteur propose *Jordanella floridae* comme sujet de laboratoire pour des études de toxicité chronique. Ce poisson atteint tôt la maturité sexuelle, commence à frayer à l'âge de 6 à 8 semaines dans des conditions optimales, et fraie en tout temps de l'année. Les adultes sont sexuellement dichromatiques. Cette espèce permet d'effectuer un essai complet de chronicité en pas plus de 3 ou 4 mois.

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THE purpose of this report is to suggest use of the flagfish (*Jordanella floridae*), a cyprinodontid fish of short generation time, as a laboratory animal and to present information concerning the laboratory culture and behavior of this species. *J. floridae* has been suggested by Foster et al. (1969) as a suitable laboratory animal for behavioral studies. It is small and requires comparatively little space, is easily maintained in the laboratory, can be spawned at any time of the year, and matures in a few weeks. The species completes a generation in 6–8 weeks, in comparison with the following periods for test species commonly used: fathead minnow (*Pimephales promelas*), 16–20 weeks; bluegill (*Lepomis macrochirus*) and brook trout (*Salvelinus fontinalis*), 2 years.

Foster et al. (1969) used a synthetic hard water successfully in a static system to rear their fish, but the species also does well in soft water. In our continuous flow system, as described by Mount and Brungs (1967), we used soft (45 ppm total hardness as $CaCO_3$) Lake Superior water, and water-volume turnover varied from 1 tank-volume turnover each week to a maximum of 6 tank-volume turnovers each 24 hr.

The adults can be maintained successfully under relatively crowded conditions even at spawning temperatures without loss or injury of the fish because of fighting. A standard 15- to 20-gal aquarium accommodates two or three territorial males and four to seven females. Further, the fish can be kept in aquaria that are completely bare, or with a gravel substrate and plants. They do well under either condition. As the species is sexually dichromatic at maturity, the investigator can easily divide the sexes as desired.

Although prolonged feeding tests have not been conducted, many foods have been tentatively evaluated on the basis of acceptance and growth of the animals. The exclusive use of dry foods results in low growth rates. A good diet for mature fish is frozen brine shrimp supplemented by a high quality dry trout food and live foods such as *Tubifex* worms or small *Daphnia*. The newly hatched fry are fed a flour-fine commercial dry food for 2 days and then newly hatched brine shrimp exclusively until, at approximately 3 weeks, they can take frozen brine shrimp.

The fish are placed in tanks sufficiently uncrowded to allow a predetermined number of males to establish territories, and spawning substrates are placed at each end. Instead of the yarn mops used by Foster et al. (1969), it is more convenient to string yarn on a frame to make a compact grid. If the yarn strands are placed close together to make a mat, the eggs can be counted readily. Synthetic fiber yarns (orlon or nylon) of any dark color can be used; bright colors, such as yellow, orange, and white, are not accepted by the fish. The water temperature for spawning should be 24–26 C, and the photoperiod should be a constant 14 hr light or more. Under these conditions mature male fish readily become territorial, one over each spawning substrate, and will vigorously drive away any intruding fish. Females usually inhabit the central and upper portions of such a tank and are permitted to approach the substrate only when ready to spawn.

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Spawning, described in detail by Mertz and Barlow (1966), may take place at any time during the light period and usually occurs several times a day. There is little, if any, discrimination by males in their acceptance of ready females. Each female has an undetermined number of spawning periods of about 14 days. In each cycle, she usually produces about 30 eggs on day 1, up to about 200 by days 7–9, and then progressively fewer. To obtain a minimum number of eggs each day, several females are used for each territorial male. This practice usually results in a daily production of 100 or more eggs from each substrate. Egg production has been maintained for approximately 3 months with no signs of a decrease in spawning activity. Eggs are collected daily by removing the spawning substrates; clean substrates are then put in. This appears to disturb the fish very little, and the male immediately takes his guarding station above the new substrate.

The egg-laden substrates, which are removed from the tanks one at a time, are placed in a shallow glass or stainless steel pan of water of the same temperature as the water from which they were removed. Eggs are then counted under a good light and are removed for hatching with a "pro-pipetter," a regulated pipette filling device. Eggs are normally hatched by assigning given numbers to egg cups and placing them on an egg rocker (Mount 1968). Hatching takes place in 4–5 days at 25 C in clean water. If fungus attacks the eggs, they are submersed daily for 5 min in a malachite green solution (4 mg/liter). This treatment appears to have no adverse effect on eggs or fry.

FOSTER, N. R., J. CAIRNS, JR., AND R. L. KAESLER. 1969. The flagfish, *Jordanella floridae*, as a laboratory animal for behavioral bioassay studies. Proc. Acad. Natur. Sci. Philadelphia 121: 129–152.

MERTZ, J. C., AND G. W. BARLOW. 1966. On the reproductive behavior of *Jordanella floridae* (Pisces: Cyprinodontidae) with special reference to a quantitative analysis of parental fanning. Z. Tierpsychol. 23: 537–554.

MOUNT, D. I. 1968. Chronic toxicity of copper to fathead minnows (*Pimephales promelas*, Rafinesque). Water Res. 2: 215–223.

MOUNT, D. I., AND W. A. BRUNGS. 1967. A simplified dosing apparatus for fish toxicology studies. Water Res. 1: 21–29.

Recent Articles on FRB Investigations Published in Other Periodicals

Until 1970, reprints of these articles had been included in an annual volume of "Studies," composed of two parts. The final binding was the 1969 Studies, Part 2, which included No. 1355. These are no longer bound and distributed. We continue, however, to number and list all articles, which henceforth may be obtained in reprint form only from the author or originating FRB establishment. The number following each title refers to the FRB establishment listed below.

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St. John's, Newfoundland

1741. Frair, W., R. G. Ackman, and N. Mrosovsky. 1972. Body temperature of *Derochelys coriacea:* warm turtle from cold water. Science 177: 791–793. (8)

1742. Healey, F. P., and J. Myers. 1971. The Kok effect in *Chlamydomonas reinhardi.* Plant Physiol. 47: 373–379. (4)

1743. Wang, R., F. P. Healey, and J. Myers. 1971. Amperometric measurement of hydrogen evolution in *Chlamydomonas.* Plant Physiol. 48: 108–110. (4)

1744. Lerman, A., and G. J. Brunskill. 1971. Migration of major constituents from lake sediments into lake water and its bearing on lake water composition. Limnol. Oceanogr. 16: 880–890. (4)

1745. Vermeer, K., and F. A. J. Armstrong. 1972. Mercury in Canadian prairie ducks. J. Wildl. Manage. 36: 179–182. (4)

1746. Drinnan, R. E. 1970. The potential for shellfish cultivation in eastern Canada. Proc. Chem. Inst. Can. Conf. Mar. Sci. Pap. 3: 2 p. (8)

1747. Ackman, R. G., S. N. Hooper, J. R. Maxwell, G. Eglinton. 1970. Implications of isoprenoid fatty acid diastercoisomerism in marine lipids and organic geochemistry. Proc. Chem. Inst. Can. Conf. Mar. Sci. Pap. 6: 16 p. (8)

1748. Idler, D. R. 1970. Marine science: a challenge to chemists. Proc. Chem. Inst. Can. Conf. Mar. Sci. Pap. 1: 15 p. (8)

1749. Dickie, L. M. 1970. Highlights from the science council study on marine science and technology in Canada. Proc. Chem. Inst. Can. Conf. Mar. Sci. Pap. 9: 11 p. (7)

1750. Whyte, J. N. C. 1970. Chemical and P. M. R. studies on glucans isolated from red alga *Rhodymenia pertusa.* Proc. Chem. Inst. Can. Conf. Mar. Sci. Pap. 14: 2 p. (2)

1751. Zitko, V. 1970. Determination of mineral oil in aquatic animals. Proc. Chem Inst. Can. Conf. Mar. Sci. Pap. 18: 19 p. (6)

1752. Sergeant, D. E. 1968. Sightings of marine mammals. ICNAF Spec. Publ. 7: 241–286. (5)

1753. Saether, O. A. 1972. *Chaoboridae,* p. 257–280. *In* Das Zooplankton, Die Binnengewasser. Pt. 1. (4)

1754. Longhurst, A., M. Colebrook, J. Gulland, R. LeBrasseur, C. Lorenzen, and P. Smith. 1972. The instablity of ocean populations. New Sci. 1: 1–4. (1)

1755. Hutzinger, O., S. Safe, and V. Zitko. 1972. Photochemical degradation of *Chlorobiphenyls* (PCBs). Environ. Health Perspect. April 1972: 15–20. (6)

1756. Ricker, W. E. 1972. Research and management. Trans. Amer. Fish. Soc. 101: 150–157. (1)

1757. Peterson, R. H. 1972. Electrical responses of goldfish cerebellum, responses to parallel fiber and peduncle stimulation. Brain Res. 41: 67–79. (6)

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The Fisheries Research Board of Canada also publishes a series of *Bulletins*, each on a specific topic. These are for sale by Information Canada, Ottawa, and remittances must be in advance, payable in Canadian funds to the order of the Receiver General of Canada. *Bulletins* are also available from local booksellers and Information Canada bookshops throughout Canada.

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